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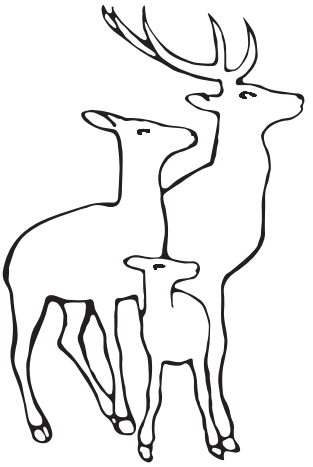

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**Review**

# Lactoferrin as treatment for iron-deficiency anemia in children: a systematic review

Devina June[1], Alvin Timothy Konstantin[1], Levina Arthauli Lumbanradja[1], Astria Aryani[2], Antoninus Hengky[2]

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## ABSTRACT

**Background.** Anemia is a common nutritional problem in children, especially those under five. Lactoferrin (Lf) as a supplement in treating iron deficiency anemia (IDA) has been studied, but its results in children have not been reviewed. This review aims to evaluate the effect of lactoferrin on children with IDA.

**Methods.** PubMed, ProQuest, EBSCO and Ovid databases were searched using a variation of keywords: lactoferrin, anemia, and children. The literature selected must be clinical trial-based in design. The years of the studies published were limited to 2012 and 2022.

**Results.** Eleven studies were included in the final systematic review, consisting of 10 randomized controlled trials (RCTs) and 1 non-randomized trial. Serum ferritin (SF) and hemoglobin (Hb) were found to be increased in groups treated with Lf or a combination of Lf and elemental iron compared to iron only or placebo supplementation. Adverse events such as constipation, vomiting, anorexia, and abdominal pain were found; particularly, a significant decrease in constipation is seen in Lf-treated groups.

**Conclusions.** This study supports Lf as a superior treatment for IDA in children regarding the improvement in hematological and iron indices and fewer adverse effects.

**Key words:** lactoferrin, iron status, anemia, children, treatment.

Anemia is one of the major global public health problems that particularly affects children aged 0-5 years and women of reproductive age. The World Health Organization (WHO) estimates that 42% of children under five are anemic.[1] When evaluating pediatric cases with anemia, a simple laboratory test such as hemoglobin (Hb) or hematocrit (Ht) can suggest the diagnosis. However, depending on the types of anemia suggested, further testing may be needed. There are several types of anemia based on the red blood cell size, including microcytic, normocytic, and macrocytic anemia. The most common cause of anemia worldwide is microcytic hypochromic anemia caused by iron deficiency.[2]

In pregnant women, iron deficiency anemia (IDA) is associated with preterm delivery, low birth weight, and decreased iron stores in infants. Throughout life, children are posed with many risk factors starting from prematurity, dietary intake of iron-fortified foods after six months, low socioeconomic status, and cow's milk introduction before age one. Thus, they have a higher risk of anemia and can lead to detrimental outcomes if not identified. Symptoms include irritability, malaise, pica, neurodevelopmental and behavioral delays, poor cognitive performance, and concentration difficulty. Supplementation with iron during infancy and preschool years is important to support physical growth, brain development, and early learning capacity. Early identification

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and intervention in anemic children can lead to an overall improvement in population health outcomes, improved physical exercise performance, and well-being that increases productivity.[3]

Lactoferrin (Lf) is a versatile glycoprotein that binds to iron and is present in both human breast milk and bovine milk. It serves various physiological purposes, such as enhancing iron absorption and possessing multiple beneficial activities, including antiviral, antibacterial, antifungal, anti-inflammatory, antiparasitic, and immunomodulatory properties.[4-6] Lf can also be found in mucosal and bronchial secretions, bile, gastrointestinal fluids, and urine. Bovine lactoferrin (bLf) has been studied for the last six decades since Lf can be extracted correctly without damaging its protein and is commercially distributed. As another transferrin, Lf's capability doubled that of transferrin. bLf also plays a part in protecting cell damage induced by oxidative stress and against iron deregulation.

An approach to treating IDA with Lf instead of iron supplementation seems promising. A meta-analysis conducted on reproductive women comparing efficacy between bLf and oral ferrous iron preparations as the treatment of IDA showed a significant increase in iron status. Lf also showed fewer gastrointestinal side effects compared to iron preparations.[7] Lf efficacy as the treatment of IDA in children is still limited and showed inconsistent results. This systematic review aims to observe the efficacy of Lf as treatment for IDA in children.

## Methods

### *Inclusion and exclusion criteria*

We included studies that investigated the effect of Lf as a treatment for IDA in children in the last ten years (2012-2022) and were available in full text (not editorials or abstracts for conferences). Documents were excluded if they were: not presented in English; case reports, case series, systematic reviews, meta-analyses, letters to editor, and book chapters; or articles with irrelevant topics.

### *Literature search strategy*

Literature searches were conducted in four electronic databases namely PubMed, EBSCO, Ovid, and ProQuest from 2012-2022. We used Boolean operators "AND/OR" with keywords/MESH terms: 1) lactoferrin, 2) anemia (iron OR anemia OR hemoglobin OR ferritin), and 3) children (children OR pediatric). Then, we combined 1), 2), and 3) with AND. Searches in other databases used similar search strategies and keywords. Separately, manual searches of literature databases and references of other articles were conducted by the authors, resulting in 7 additional articles for consideration.

### *Study selection and data extraction*

The searches yielded 2503 results. Following the PRISMA guidelines, we eliminated all the duplicates and a total of 2402 records were divided between authors equally. Subsequently, five authors (D.J., A.T., A.A., L.A.L, A.H.) screened the collected articles independently based on the title and abstract. The remaining articles were evaluated in full-text according to the inclusion and exclusion criteria. All the authors discussed and reached the final eleven selected articles included in this systematic review through voting (Fig. 1).

### *Quality assessment*

We used the Revised Cochrane risk-of-bias (RoB 2) tool to critically appraise studies with randomized-controlled trial designs and ROBBINS-I for non-randomized controlled studies. Disagreements between reviewers were resolved by discussion. The risk of bias summary of the included studies is detailed in (Fig. 2).

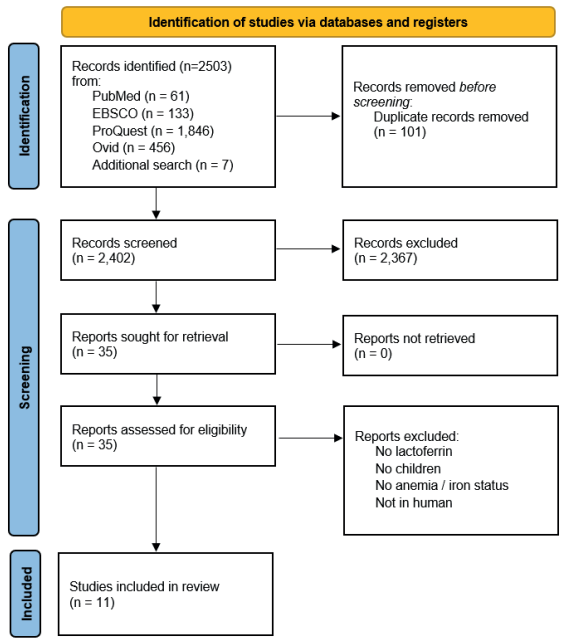



**Fig. 1.** PRISMA flow diagram.

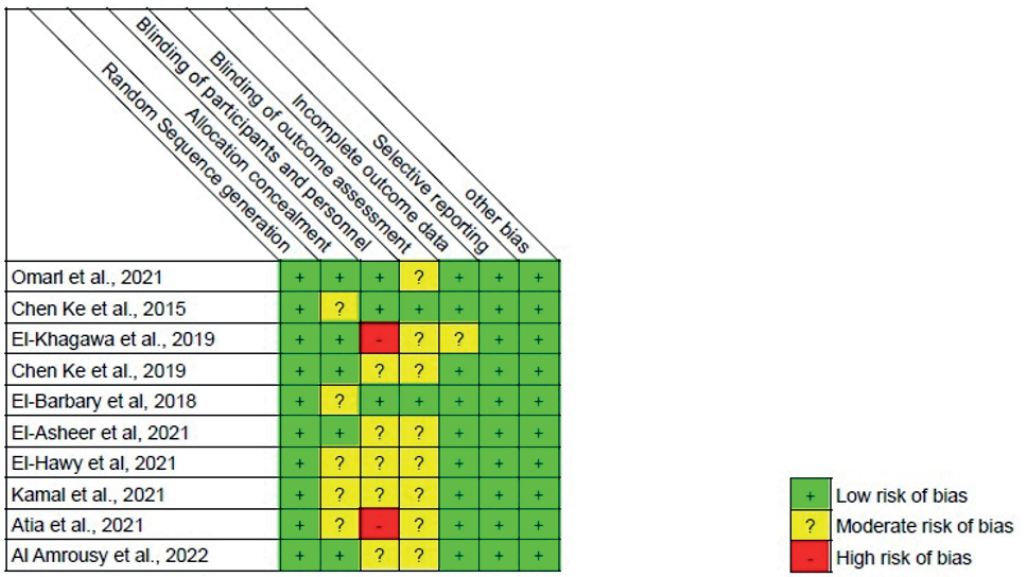
## Results

### *Literature search*

After the removal of duplicates, 2402 records resulted from the initial search (Fig. 1). Screening by title and abstract selected 35 articles eligible for full-text analysis. Twenty-four records were excluded with reasons, resulting in a total of 11 records included in the systematic review.

### *Characteristics of the included studies*

We found data on Lf and IDA (n=11). Studies were published between 2012 and 2022 and carried out in Egypt (n=8), China (n=2), and Germany (n=1). There was considerable heterogeneity in recruitment protocol interventions. Seven were randomized control trials, and one was a non-randomized trial.




| Study | Pre-intervention | | At Intervention | Post-intervention | | | | Overall risk of bias |
|---|---|---|---|---|---|---|---|---|
| Author | Bias due to confounding | Bias in selection of participants into the study | Bias in classification of interventions | Bias due to deviations from intended interventions | Bias due to missing data | Bias in measurement of outcomes | Bias in selection of the reported result | low/moderate/serious/ critical |
| El-Latef et al., 2019 | Low | Moderate | Moderate | Low | Low | Low | Low | Low |

**Fig. 2.** Risk of bias assessment.

Treatment duration ranged from 1 month to 10 months. The studies predominantly enrolled populations <18 years old with a total of 1050 participants.

***Reviewed article summaries***

The characteristics of the studies assessed are presented in Table I.[6,8-17] All studies investigated the effect of Lf as a treatment for IDA in children. There were various types and dosages of Lf used as the intervention group. Most studies used 100 mg oral Lf as the intervention group[6,8,9,11-17], whereas few combined Lf and elemental iron.[10,14-16] The therapy was administered orally in all studies. The result compared hematological and iron indices between the Lf group and the control group and other intervention groups. Numerical results of the studies assessed are presented in Table II.[6,8-17]

In a study by Omar et al.[6], 70 children aged 1-10 years with cerebral palsy (CP) and IDA were enrolled into 2 groups: oral Lf and iron polymaltose complex (IPC) as the control, were administered for 1 month. There was an increase in adjusted mean changes of Hb and Serum ferritin (SF) levels in the Lf group. The adverse effect rate was lower in the Lf group, commonly constipation.

Mohamed et al.[8] enrolled 30 children aged between 6 months and 5 years admitted by prolonged chest infection with IDA, 15 with 100 mg Lf and 15 with no intervention, for 1 month. There was no significant difference in Hb, Ht, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), changes. However, total iron binding capacity (TIBC) in the Lf group was higher than in the control group after treatment.

Eighty children with IDA who suffer from inflammatory bowel disease were enrolled in a study by El-Amrousy et al.[9]. Participants were divided into the ferrous sulfate (FS) group (6 mg/kg/day) and Lf group (Lf 100 mg/day) and treatment was continued for 3 months. Lf significantly increased Hb, Serum Iron (SI), transferrin, and SF compared to FS. Lf significantly decreased interleukin-6 (IL-6) and hepcidin levels. In the FS group, 18 patients (46.2%) experienced gastrointestinal side effects (abdominal pain, nausea, and diarrhea).

Ke et al.[10] conducted 2 studies in China in both 2015 and 2020. The 2015 study was conducted with 213 breastfed infants aged 4 to 6 months divided into 2 groups: Lf 38 mg/100g with iron 4 mg/100g and iron element 4 mg/100g as the control for 3 months. There was a statistically significant increase of Hb, red blood cells (RBCs), MCH concentration (MCHC), SF, and SI after treatment in Lf with iron. The 2020 study[12] was conducted on 105 previously breastfed children who were weaned and formula-fed at 6 to 9 months. They were divided into 3 groups for 3 months: formula fortified with Lf 38 mg/100 g, Lf 76 mg/100 g, and without Lf. The Hb level in Lf 76 mg/100 g was significantly higher than the other groups after 3 months. No significant difference in levels of SF, serum transferring receptor (sTfR), sTfR-SF index (sTFR-F index), and TIBC among the infants in the three groups after intervention, and no important adverse effects were observed.

The study by El-Khawaga et al.[11] included 94 children with IDA, aged 6-12 years old, divided into 2 groups: 47 were given 100 mg of oral bLf and 47 were given iron at 6 mg/kg/day for 1 month. This study found that there were significant increases in Hb, RBC, MCHC, SF, and SI after treatment with Lf when compared to the elemental iron group.

A study of 52 neonates who were admitted to the neonatal intensive care unit (NICU) from birth to day 30 of life in Egypt by El-Barbary et al.[13] found a significantly higher SF, Hb, Ht, and MCV in the intervention group who were given Lf 100 mg/day compared to the control group after 1 month.

A study by El-Asheer et al.[14] enrolled 96 children

**Table I.** Selected studies' characteristics and adverse effects.

| No. | Author, Year, Country | Study population | Treatment duration | Adverse Effects |
|---|---|---|---|---|
| 1 | Omar et al.[6], 2021, Germany | 70 children aged 1-10 years with Cerebral Palsy and IDA | 1 month | Adverse effects were lower in Lf groups, but only constipation is significant (p-value=0.049). |
| 2 | Mohamed et al.[8], 2019, Egypt | 30 children aged between 6 months and 5 years admitted by prolonged chest infection with IDA | 1 month | No data |
| 3 | El-Amrousy et al.[9], 2022, Egypt | 80 IDA children with IBD | 3 months | In the ferrous sulfate group, 18 patients (46.2%) experienced gastrointestinal side effects |
| 4 | Ke et al.[10], 2015, China | 213 infants aged 4 to 6 months | 3 months | No data |
| 5 | El-Khawaga et al.[11], 2019, Egypt | 94 children with IDA aged 6-12 years old | 1 month | No data |
| 6 | Ke et al.[12], 2020, China | 105 previously breastfed but weaned and formula-fed at 6 to 9 months | 3 months | No important adverse events or side effects in each intervention group were observed |
| 7 | El-Barbary et al.[13], 2018, Egypt | 52 neonates who were admitted to NICU from birth to day 30 of life | 1 month | No data |
| 8 | El-Asheer et al.[14], 2021, Egypt | 96 children above 2 years old with IDA | 10 months | There were significantly lower adverse events in Lf group compared to combination group and iron group |
| 9 | El-Hawy et al.[15], 2021, Egypt | 120 children with IDA aged 1-18 years old | 1 month | Side effects of drugs were significantly higher in Lf with iron group than FeBC group (p-value=0.007) and Lf group (p-value<0.001) |
| 10 | Kamal et al.[16], 2021, Egypt | 150 children aged above 2 years with IDA | 3 months | No data |
| 11 | Atia et al.[17], 2021, Egypt | 40 obese children and adolescents aged between 6-18 years with IDA | 3 months | Children in the lactoferrin therapy group experienced fewer side effects |

IBD: inflammatory bowel disease, IDA: iron deficiency anemia.

**Table II.** Changes (mean ± SD) in hematological parameter and iron indices among included studies.

| Author, Year | Population Age | Study Group | Hb (g/dL) | | p-value | SF (ng/mL) | | p-value | SI (µg/dL) | | p-value | TIBC (µg/dL) | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Before | After | | Before | After | | Before | After | | Before | After | |
| Omar et al.[6], 2021 | 1-10 years | IPC (6 mg/kg/d) | 8.74 ± 1.85 | 9.48 ± 2.24* | <0.001 | 7.27 ± 5.78 | 10.32 ± 4.78* | 0.080 | 15.59 ± 10.24 | 24.41 ± 16.02* | 0.247 | 435 ± 100.31 | 358 ± 110.98* | 0.739 |
| | | Lf (100 mg bid) | 8.89 ± 1.94 | 9.7 ± 1.81* | | 6.35 ± 5.69 | 11.99 ± 11.56* | | 13.41 ± 11.39 | 24.25 ± 16.9* | | 430 ± 115.25 | 352 ± 106.71* | |
| Mohamed et al.[8], 2019 | 6 months-5 years | Water | 9.3 ± 0.92 | 9.4 ± 1.09x | N/A | 15.0 | 4.65* | 0.37 | 34.6 ± 8.9 | 39.4 ± 11.3x | 0.47 | 284.1 ± 54.2 | 301.3 ± 42.1 | 0.017 |
| | | Lf (100 mg qd) | 9.7 ± 0.98 | 9.8 ± 1.02x | | 20.8 | 4.50* | | 25.4 ± 9 | 36.4 ± 11* | | 263.0.7 ± 9 | 355 ± 68.7* | |
| El-Amrousy et al.[9], 2022 | 5-18 years | FS (6 mg/kg/d) | 9.2 ± 1.6 | 10.8 ± 0.49* | 0.010 | 21.3 ± 6 | 32.2 ± 5.4* | 0.006 | 38.8 ± 3.8 | 44.7 ± 3.9* | 0.001 | 392 ± 33 | 283 ± 38* | 0.7 |
| | | Lf (100 mg qd) | 9.1 ± 1.2 | 11.9 ± 1.7* | | 20.4 ± 6.5 | 38.4 ± 6.1* | | 38.6 ± 3.8 | 49.5 ± 3.9 | | 398 ± 28 | 286 ± 27* | |
| Ke et al.[10], 2015 | 4-6 months | Iron (4 mg/100 g) | 10.98 ± 1.19 | 11.69 ± 1.31* | <0.001 | 22.7 ± 13.5 | 31.6 ± 18.4* | <0.001 | N/A | | | 471 ± 24 | 526 ± 55* | <0.001 |
| | | Lf (38 mg/100 g) | 11.17 ± 1.21 | 12.55 ± 1.54* | | 25.4 ± 14.1 | 44.7 ± 17.2* | | | | | 475 ± 22 | 612 ± 78* | |
| El-Khawaga et al.[11], 2019 | 6-12 years | Iron (6 mg/kg/d) | 9.6 ± 0.66 | 10.2 ± 0.7 | <0.001 | 16.6 ± 7.4 | 24.8 ± 9.4 | <0.001 | 41.9 ± 8.9 | 63.0 ± 14.7 | 0.038 | 380 ± 47 | 320 ± 47 | 0.692 |
| | | Lf (100 mg qd) | 9.7 ± 0.49 | 10.84 ± 0.59 | | 14.9 ± 7.46 | 40.3 ± 18.3 | | 39.2 ± 8.8 | 69.6 ± 14.3 | | 390 ± 42 | 316 ± 46 | |
| Ke et al.[12], 2020 | 6-9 months | Iron (4 mg/100 g) | 10.29 ± 0.8 | 11.65 ± 0.8* | <0.006 | 8.4 ± 1.2 | 25.6 ± 3.7* | 0.374 | N/A | | | 412 ± 35 | 662 ± 51* | 0.193 |
| | | Iron + Lf (38 mg/100 g) | 10.08 ± 0.8 | 11.66 ± 0.6* | | 8.9 ± 1.3 | 26.9 ± 4.4* | | | | | 425 ± 29 | 653 ± 47* | |
| | | Iron + Lf (76 mg/100 g) | 10.51 ± 0.81 | 12.14 ± 0.51* | | 8.2 ± 1.2 | 26.2 ± 3.1 | | | | | 423 ± 22 | 678 ± 63* | |
| El-Barbary et al.[13], 2018 | 0 days (newborn) | Water | 15.2 ± 2.0 | 11.8 ± 7.3* | <0.001 | 341.2 ± 60.9 | 249.4 ± 72.7* | p<0.001 | N/A | | | N/A | | |
| | | Lf (100 mg/day) | 16.4 ± 2.8 | 15.7 ± 1.5x | | 327.1 ± 45.5 | 382.3 ± 89.8x | | | | | | | |

Lf, lactoferrin; Hb, Hemoglobin; SF, Serum Ferritin; SI, Serum Iron; TIBC, Total Iron Binding Capacity; qd, once a day; bid, twice a day; IPC, Iron Polymaltose Complex; FeBC, Iron Bisglycinate Chelate; FG, Ferrous Gluconate

*significant result between before and after intervention within one group

xnon-significant result between before and after intervention within one group

p: p-value for the result after intervention between two study group; p1: p-value group 1 vs 2; p2: p-value group 2 vs 3; p3: p-value group 1 vs 3; p4: p-value group 2 vs 4.

**Table II.** Changes (mean ± SD) in hematological parameter and iron indices among included studies.

| Author, Year | Population Age | Study Group | Hb (g/dL) | | p-value | SF (ng/mL) | | p-value | SI (µg/dL) | | p-value | TIBC (µg/dL) | | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Before | After | | Before | After | | Before | After | | Before | After | |
| El-Asheer et al. [14], 2021 | 2-15 years | Iron (6 mg/kg/d) | 9.95 ± 0.56 | 10.24 ± 0.57* | p1<0.001 | N/A | | | 22.84 ± 4.10 | 28.94 ± 5.05* | p1<0.001 | N/A | | |
| | | Lf (100 mg qd) | 9.95 ± 0.87 | 11.06 ± 0.96* | p2=0.368 | | | | 24.17 ± 2.67 | 42.79 ± 6.14* | p2=0.089 | | | |
| | | Iron + Lf (same dose as above) | 10.05 ± 0.75 | 11.24 ± 0.71* | p3<0.001 | | | | 24.09 ± 3.55 | 45.67 ± 8.42* | p3<0.001 | | | |
| El-Hawy et al. [15], 2021 | 1-18 years | FeBC (0.75 mg/kg/d) | 10.08 ± 0.44 | 11.93 ± 0.38 | p1<0.001 | 6.68 ± 1.36 | 20.91 ± 3.33 | p1<0.001 | 36.97 ± 5.73 | 64.34 ± 9.94 | p1<0.001 | 431.3 ± 30.39 | 371.0 ± 25.18 | N/A |
| | | Lf (100 mg qd) | 10.39 ± 0.4 | 11.06 ± 0.45 | p2<0.001 | 7.17 ± 0.48 | 7.51 ± 1.68 | p2<0.001 | 38.60 ± 5.68 | 45.07 ± 6.03 | p2<0.001 | 438.7 ± 19.92 | 390.27 ± 33.93 | |
| | | Lf (100 mg) + Iron (30 mg), qd | 10.29 ± 0.51 | 11.86 ± 0.36 | p3=0.879 | 7.46 ± 2.18 | 20.60 ±2.91 | p3=0.965 | 37.63 ± 5.81 | 61.73 ± 8.48 | p3=595 | 438.6 ± 27.72 | 375.40 ± 26.41 | |
| | | IPC (6 mg/kg/d) | 10.26 ± 0.43 | 11.63 ± 0.33 | p4<0.001 | 7.62 ± 1.9 | 18.57 ± 1.99 | p4<0.001 | 37.27 ± 6.30 | 58.27 ± 7.25 | p4<0.001 | 437.6 ± 17.46 | 374.40 ± 31.26 | |
| Kamal et al. [16], 2021 | Above 2 years | Iron (6 mg/kg/d) | 8.6 ± 0.8 | 11.2 ± 0.9 | <0.001 | 5.7 ± 7.2 | 11.4 ± 4.16 | 0.-26 | 18 ± 7 | 38 ± 12 | <0.001 | 700 ± 130 | 490 ± 120 | <0.001 |
| | | Lf (100 mg bid) | 8.2 ± 0.97 | 9.7 ± 1.2 | | 4.1 ± 1.2 | 7.7 ± 3.6 | | 32 ± 9.6 | 32 ± 18 | | 720 ± 170 | 530 ± 150 | |
| | | Lf (100 mg) + FG, bid | 8.4 ± 0.83 | 11.6 ± 0.7 | | 6.0 ± 5.1 | 19.6 ± 4.45 | | 22 ± 7 | 41 ± 29 | | 690 ± 190 | 410 ± 80 | |
| Atia et al. [17], 2021 | 6-18 years | Iron (6 mg/kg/d) | 9.9 ± 0.48 | 11.67 ± 0.33* | <0.001 | 19.67 ± 6.81 | 29.95 ± 8.40* | 0.018 | 42 ± 14 | 60 ± 17.1* | 0.016 | 390 ± 38.7 | 368 ± 29.7 | 0.259 |
| | | Lf (100 mg qd) | 9.8 ± 0.49 | 12.48 ± 0.66* | | 16.88 ± 7.96 | 38.33 ± 12.65* | | 46 ± 14 | 72 ± 12.4* | | 413 ± 45.8 | 354 ± 43.5 | |

Lf, lactoferrin; Hb, Hemoglobin; SF, Serum Ferritin; SI, Serum Iron; TIBC, Total Iron Binding Capacity; qd, once a day; bid, twice a day; IPC, Iron Polymaltose Complex; FeBC, Iron Bisglycinate Chelate; FG, Ferrous Gluconate

*significant result between before and after intervention within one group

xnon-significant result between before and after intervention within one group

p: p-value for the result after intervention between two study group; p1: p-value group 1 vs 2; p2: p-value group 2 vs 3; p3: p-value group 1 vs 3; p4: p-value group 2 vs 4.

above 2 years old with IDA and divided them evenly into 3 groups evenly: Group I (Lf 100 mg/day), Group II (iron 6 mg/kg/day), and Group III (Lf 100 mg/day with iron 6 mg/kg/day) for 10 months. There was a significant difference in RBCs, Hb, Ht, MCV, MCH, and SI after treatment in the Lf and Lf with the iron group compared to the iron group only, but there was no significant difference between Groups I and III. There were statistically significant lower adverse events in the Lf group (9.3%) compared to the Lf with iron group (15.1%) and iron only group (33.2%). The adverse events included constipation, diarrhea, anorexia, and gastric upsets.

El-Hawy et al.[15] included 120 children with IDA aged 1-18 years old and divided them evenly into 4 groups: iron bisglycinate chelate (FeBC): 0.75 mg/kg/d; 100 mg Lf; 100 mg Lf and 30 mg iron combination; and IPC 6 mg/kg/d for 1 month. There was no significant difference between the FeBC group and the Lf with iron group regarding CBC and iron profile. Hb, MCH, SI, and SF were significantly higher in the Lf with iron group than the IPC group and Lf only group. Side effects of drugs were significantly higher in Lf with iron group and IPC group than the FeBC group and Lf group. The adverse events included are constipation and black stool.

A study by Kamal et al.[16] with a population of 150 children aged above 2 years with IDA were divided equally into 3 groups for 3 months: Lf 100 mg, Lf with FG, and ferric hydroxide 6 mg/kg/day. There were significant elevations in Hb, MCV, MCH, SF, SI, and transferrin saturation and lower TIBC in Lf with FG compared to other groups.

Atia et al.[17] enrolled 40 obese children and adolescents aged between 6-18 years with IDA and divided them into 2 groups, a group that received Lf 100 mg/day, and a group that received ferric hydroxide 6 mg/kg/day for 3 months. In the Lf group, significant elevations in Hb, MCV, MCH, SF, SI, transferrin saturation, and lower TIBC were seen. Lower serum hepcidin and IL-6 were also found in the Lf therapy. Children in the Lf therapy group also experienced fewer side effects.

### *Lactoferrin and iron indices*

Groups given Lf-containing regiments generally have higher iron indices compared to the control group, either with iron supplementation or no supplementation at all. Among the iron indices (SF, SI, and TIBC), SF was the common iron indice that increased after Lf supplementation. SF level was found to change significantly at the end of most studies assessed. SI levels were significantly higher in the studies by Ke et al.[10] and El-Khawaga et al.[11] after Lf supplementation. In the study by El-Hawy et al.[15], SF and SI were increased further after FeBC and the combination of Lf with iron supplementation. SF was higher after the treatment of Lf compared to the baseline but did not differ from other intervention groups in Ke et al.[12] and Mohamed et al.[8] In the latter study, Lf did not increase iron indices compared to the control group in participants with prolonged chest infections, but TIBC was found to be increased in the intervention group after the treatment.

### *Lactoferrin and hematological parameters*

All studies evaluated the association between Lf supplementation and hematological parameters in children; those parameters included Hb, Ht, MCV, MCH, MCHC, and RBC, with Hb as the most common parameter increasing after intervention. Five studies showed a significant increase in Hb in the Lf group compared to the control group. In the study by Ke et al.[12], Lf supplementation showed a dose-response relationship, with higher increment in Hb levels along with a higher concentration of Lf. The study by El-Asheer et al.[14] showed no significant difference between groups treated with Lf and the combination of Lf and iron.

Three studies showed a significant increase in RBCs. Other parameters in some of the studies, such as hematocrit, MCV, MCH, and MCHC,

also showed significant increases. Although, a study by Mohamed et al.[8] showed no significant difference in Hb, Ht, MCV, and MCH changes. Ke et al.[10] only showed an increase in Hb levels after intervention.

### *Adverse effects of lactoferrin*

Various studies found that Lf therapy had fewer documented adverse effects than FS and combination therapy for IDA. The most common adverse events recorded were constipation, diarrhea, vomiting, anorexia, abdominal pain, and black stool. Three studies showed a significantly lower incidence of constipation in Lf groups than in the FS and combination groups. Omar et al.[6] showed that adverse effects were lower in Lf groups than FS and combination groups, but only constipation was significant. This finding is consistent with results from El-Asheer et al.[14] that showed adverse events in the Lf group (9.3%), FS group (33.3%), and Lf + FS group (16.1%). Similar findings were also seen in the study by El-Amrousy et al.[9] regarding gastrointestinal side effects; abdominal discomfort was experienced in one patient in the Lf group (2.5%), whereas abdominal pain, diarrhea, nausea, and vomiting were reported in the FS group (46.2%).

### *Additional findings*

One study by El-Barbary et al.[13] in the NICU found that Lf supplementation can help reduce RBC transfusion needs in patients. This study also found that the group given Lf reached full enteral feeding more rapidly, gained more weight at one month of age, and had a shorter length of stay in the NICU compared to the placebo group. This study found no significant decrease in mortality in patients receiving Lf. However, a decrease in mortality rate was observed in the Lf group (0% in the Lf group and 19.2% in no Lf group). The study by Ke et al.[10] found the levels of weight, weight by age, and weight by height of infants (4 to 6 months) given fortified Lf were significantly higher than those of infants in the control group, but a subsequent study by Ke et al.[12] in 2020 found no significant difference in anthropometric indices between the Lf and no Lf group in 6 to 9 month infants. Moreover, significant increases in compliance were found in the Lf group and combination group compared with the iron group in a study by El-Asheer et al.[14].

## Discussion

During the last decade, bLF's role as prophylaxis and treatment of anemia in pregnant women has been conducted in several studies producing promising results. The iron regulatory function of Lf has been confirmed in pregnant women in many clinical trials, including randomized ones.[18] These findings showed an attractive alternative to oral FS, the current go-to therapy option for IDA. Multiple studies found comparable results of lower adverse effects, significantly improved number of RBCs, Hb, serum iron (SI), and SF concentrations compared to those detected in pregnant women suboptimally treated with FS.[19] This systematic review reported similar results in the pediatric population, showing promise for Lf as an option for treatment for children with IDA.

Lf is a non-haem protein that binds to iron. Its structure and chemical composition resembles serum transferrin, which is responsible for transporting iron in the bloodstream. This protein is produced by epithelial cells of the mucosal tissues and is present in various secretions, including saliva, tears, nasal and bronchial secretions. Additionally, it is notably abundant in milk.[11] Most studies evaluated hematological parameters after intervention with Lf, and five showed significant increases, especially in Hb. Followed by other parameters that showed increases such as RBC, hematocrit, MCV, MCH, and MCHC. Lf effectively enhances the absorption of iron by exhibiting a strong affinity to two irons. When Lf binds to its receptor on intestinal cells, enabling the Lf molecule to enter the cell. Subsequently, iron is released inside the intestinal cell and transported to the bloodstream through transferrin.[9]

Lf did not improve iron or hematological parameters in the study by Mohamed et al.[8], which involved prolonged chest infections. This might suggest that prolonged inflammation might affect Lf. Inflammatory cytokines such as IL-6 decreases intestinal iron absorption by promoting the gene transcription of hepcidin and decreasing the gene expression of ferroportin.[9] However, Lf was found to be significant in increasing hematological and iron parameters in a study by El-Amrousy et al.[9] which involved participants with inflammatory disease. Elemental iron may catalyze reactions that generate oxygen-free radicals that can exacerbate inflammation and worsen the symptoms of IBD, therefore Lf might offer a better alternative. Atia et al.[17] found that serum IL-6 and hepcidin decreased significantly after Lf therapy. A previous study by Paesano et al.[19] found that bLf decreased serum IL-6 and hepcidin significantly, which mitigates ferroportin downregulation, thus permitting transport of iron from tissue to blood and restoring physiological values of SI and SF. This might help improve anemia in chronic diseases, however, it still needs to be elucidated further.

Lf's ability to enhance iron uptake and improve hematological parameters consequently also lowers transfusion needs, hastens the duration of hospitalization, and lowers mortality rates in neonates patients as found in a study by El-Barbary et al.[13] Besides Lf's effect on iron and hematological parameters, a lower mortality rate in theLf group may be related to its immunomodulatory characteristics. Lf is known to be able to downregulate pro-inflammatory cytokines in intestinal epithelial cells, suppress free radical activity and decrease the level of oxidative products in infants. Aligning with this finding, other studies show diminished severity and longitudinal prevalence of diarrhea in children receiving Lf and also a significantly lower number of lower respiratory tract illnesses in the Lf group, which both diseases are two leading causes of death in children under five.[20,21]

In anthropometric measurement, the effect of Lf supplementation showed different results, being specifically significant in infants 4 to 6 months of age and insignificant in 6 to 9 months of age.[10,12] Lf potently promotes bone growth by stimulating the proliferation and differentiation of primary osteoblasts.[22] However, the contrary results could be explained by the normal growth rate of infants, which is not as rapid during the second half of the first year of life.

Lf significantly has fewer gastrointestinal side effects than iron supplementation.[6,14,15] It is a natural compound with limited proven side effects, a protein that accompanies life, from human milk Lf to treatment for chronic diseases in adults.[23-25] El-Asheer et al.[14] showed that a longer duration of treatment (10 months) with Lf for combination therapy also has significantly fewer adverse effects than iron therapy. Lf has been approved as a Generally Recognized as Safe (GRAS) compound by the US FDA and as a dietary supplement by the European Food Safety Authority.[26,27] Powers et al.[28] explained in their study that barriers to oral iron administration in children with IDA are its side effects and poor taste. Hence, as explained before, the Lf group could achieve better compliance with fewer adverse effects from its consumption.

### *Strength and limitation*

Our systematic review has several strengths. This is the first systematic review regarding Lf as a treatment for IDA in the pediatric population. Our systematic review is the most recent on this topic, with an adequate number of studies, including eleven articles, ten RCTs, and one non-randomized study. All studies included in this review are experimental studies and have been assessed using the ROBBINS-I and RoB-2 Cochrane risk of bias tool showing a low risk of bias.

However, findings among studies showed high heterogeneity due to different age spans among samples, different intervention arms, and

different outcome measure parameters. Studies included have a relatively short duration, mostly one to three months, and only one study was conducted for ten months.

In conclusion, this study provides evidence to support Lf as a superior supplement to improve serum hematological and iron indices with fewer adverse effects in children. Lf as an immune modulator affects iron homeostasis via a lactoferrin-dependent signal transduction mechanism, which explains the functions of Lf in the regulation of iron absorption. Further mechanism studies for the functions of Lf in bone growth are warranted to explore more.

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ATK, AA; data collection: DJ, ATK, AA, LAL, AH; analysis and interpretation of results: DJ, LAL, AH; draft manuscript preparation: DJ, ATK, AA, LAL, AH. All authors reviewed the results and approved the final version of the manuscript.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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**Original Article**

# Outcomes of newborns with tracheostomy: single center experience

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## ABSTRACT

**Background.** Babies with severe bronchopulmonary dysplasia (BPD) are discharged with the support of a home-type mechanical ventilator, after opening a tracheostomy. In addition, although rare, tracheostomy is required in the neonatal period in congenital airway malformations. Early tracheostomy is appropriate to prevent complications due to prolonged intubation.

We aimed to find the appropriate time for tracheostomy by examining the tracheostomy opening and closing times, complications and demographic characteristics of the patients, who were hospitalized and underwent tracheostomy in our neonatal intensive care unit.

**Methods.** This retrospective study involved infants admitted to the neonatal intensive care unit between January 2014 and 2019 and discharged following tracheostomy. Information acquired from hospital data was enrolled. The protocol was registered with ClinicalTrials.gov identifier NCT04497740.

**Results.** Twenty-six neonates with median 27.5 weeks gestational age and birth weight 885 gr were enrolled in the study. The mean opening time for tracheostomy was 54 ± 24 days, and the postmenstrual age (PMA) was 36 ± 3 weeks. The mean time to closure of tracheostomy in newborns with a tracheostomy was 387 ± 164 days. The duration of accidental decannulation developed as an early complication in 8 patients was mean 11 ± 8 days. Aspiration pneumonia in 2, subglottic stenosis in 5, accidental decannulation in 2, suprastomal collapse in 7, tracheocutaneous fistula in 8 and granulation tissue in 2 patients were found to be late complications, which occurred within median 90 days.

**Conclusions.** If there is no evidence that breathing has improved and the patient is still using a mechanical ventilator at high pressures and high oxygen concentration, a tracheostomy placement should be considered within two months.

**Key words:** newborn, neonatal tracheostomy, extremely low birth, premature, congenital airway malformations.

The chances of survival of premature babies with an extremely low birth weight have increased in recent years along with comorbidities. One of the issues regarding premature babies is bronchopulmonary dysplasia (BPD). Despite early surfactant therapy, optimizing ventilator strategies, and increased use of noninvasive positive pressure ventilation, BPD continues to be a complication of premature births.[1-3]

It is a well-known fact that newborns with severe BPD have to be ventilated by invasive mechanical ventilation. These cases require tracheostomy placement for prolonged ventilation.[4] Besides BPD, term babies who were born with congenital airway malformations, upper airway compression due to any lesion

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in the neck or neuromuscular diseases need long term ventilation. Even if these babies have minimal ventilatory needs, tracheostomy plays a vital role to ensure airway patency in many of these conditions.[5] Moreover, tracheostomy enables long-term, stable delivery of adequate positive airway pressure that may reduce breathing and promote growth. The minimization of agitation and airway injury caused by conventional endotracheal tubes, greater ease of movement, oral feeding and safer participation in developmental therapy may be additional benefits of a tracheostomy.[6]

Several studies focus on the surgical indications, complications, and techniques used for pediatric tracheostomy whereas few studies focus on the complications and long-term clinical outcomes for infants that require tracheostomy placement and prolonged mechanical ventilation.[7-9] However, the studies in the literature regarding tracheostomy timing and decannulation procedure in newborns or infants are limited.[4,10,11] In addition, the opening time of a tracheostomy varies between NICUs. Rather, it depends on the clinician's decision.

We aimed to find the appropriate time for tracheostomy by examining opening and closing times, complications and demographic characteristics of the patients who were hospitalized and underwent tracheostomy in our neonatal intensive care unit (NICU).

## Material and Methods

This study was designed as a retrospective study. The records of all newborns admitted to the NICU and underwent a tracheostomy at our hospital between January 2014 and 2019 were reviewed. This study was approved by the Hacettepe University Non-Interventional Ethical Committee (2020/11-28). Premature newborns with a severe BPD and term newborns diagnosed with congenital airway malformations or different kind of diseases requiring tracheostomy were involved in our study. The National Institute of Child Health and Human Development (NICHD), NHLBI and Office of Rare Diseases (ORD) proposed the current (NIH consensus) definition of BPD, "Infants born < 32 weeks, who require supplemental oxygen for at least 28 days and at 36 weeks postmenstrual age (PMA)". The definition further stratified the disease into mild (breathing room air at 36 weeks PMA or discharge, whichever comes first), moderate (need for <30% oxygen at 36 weeks PMA or discharge, whichever comes first), severe (need for ≥30% oxygen and /or positive pressure [positive pressure ventilation (PPV) or nasal continuous positive airway pressure (NCPAP)] at 36 weeks PMA or discharge, whichever comes first).[1] The NICHD Neonatal Research Network has proposed a severe BPD definition using a severity scale that is based on the use of positive pressure at 36 weeks PMA instead of supplemental oxygen. Infants were classified as no BPD (no support), grade 1 (nasal cannula ≤2 L/min), grade 2 (nasal cannula >2 L/min or non-invasive positive airway pressure), or grade 3 (invasive mechanical ventilation).[2] Patients' demographic information such as antenatal follow-up data, maternal diseases, birth weight, gestational age, delivery type, as well as invasive or non-invasive mechanical ventilation times during hospitalization in our unit were recorded. In addition, tracheostomy opening time and tracheostomy closure time at follow-up were added.

There are approximately 1000 births per year in our center and there are perinatology, pediatric surgery and many other surgical branches. In addition to high-level surgical procedures, we have a fourth-level neonatal intensive care unit that can be perform extracorporeal membrane oxygenation (ECMO). Tracheostomy procedures are performed especially by physicians specialized in pediatric and neonatal ear, nose and throat surgery (ENT).

All tracheostomies were performed through an endotracheal tube in the operating room by experienced otolaryngologists. All surgeons followed the same surgical technique. Patients were positioned with a shoulder roll and annular

head support in place. Cervical landmarks were identified and a midline horizontal incision was made with a knife along the skin. Colorado-tipped electrocautery was used to remove subcutaneous and perimuscular fat and dissect down to the anterior trachea in the midline. Lateral dissection was avoided. A skin hook was placed under the cricoid cartilage to elevate the trachea. A vertical incision was made between the second and third tracheal rings with an 11-blade scalpel. Bilateral stop sutures were placed through a vertical incision. The addition of maturation sutures from skin to trachea forms a mature stoma which, in conjunction with the use of stay sutures, allows easier replacement of the tube if accidentally displaced. The appropriately sized tracheostomy tube was then placed. The anesthesiologist then controlled for significant air leaks, even air intake and whether adequate oxygenation and ventilation could be maintained. The tracheostomy tube was then fixed in place with nylon sutures and tracheal ties. All patients underwent postoperative chest radiographs to evaluate tracheostomy tube placement and to determine whether there were any changes in the lungs after the procedure. Tracheostomy tubes were changed in the NICU by an otolaryngologist between postoperative days 5 and 7.

In addition, early and late complications encountered in the follow-up of patients with tracheostomy were also recorded in our study.

Before decannulation, airway examination via laryngo-bronchoscopy is the standard of care.

Data were analyzed using "Statistical Package for Social Sciences for Windows 27.0." and were tested for normality with the Shapiro Wilk W-test; they were expressed as mean (SD) and median (minimum- maximum) as appropriate. In the analysis, p values less than 0.05 were accepted as statistically significant. The protocol was registered with ClinicalTrials.gov identifier NCT04497740.

## Results

A total of 26 newborn infants who underwent a tracheostomy between January 2014 and 2019 were enrolled in our study. Demographic data such as gestational age, birth weight, gender, and maternal diseases are listed in Table I.

20 of the newborns who underwent tracheostomy were followed up with the diagnosis of bronchopulmonary dysplasia. The newborns median gestational ages were 26 (24-32) weeks and median birth weights were 790 grams (420- 2015). 12 (60%) of these newborns used antenatal steroids.

**Table I.** Demographic data (n=26).

| | |
|---|---|
| Gestational age, weeks* | 27.5 (24-39) |
| Birth weight, gram* | 885 (420- 3160) |
| Weight for gestational age (AGA/SGA/LGA) | 23 (88.5)/ 3 (11.5)/ 0 |
| APGAR 1. /5. min* | 6 (2-8) / 7 (4-9) |
| Delivery type (Cesarean section) | 23 (88.5) |
| Gender (male) | 17 (65) |
| Antenatal steroid | 13 (50) |
| Mother's diseases | |
| Gestational diabetes | 2 (8) |
| Preeclampsia | 8 (31) |
| Urinary tract infection | 1 (4) |
| Chorioamnionitis | 1 (4) |

Expressed as n (%) unless indicated otherwise. * Median (min- max)

**Table II.** Morbidities and respiratory support (n=26).

| Morbidities | n (%) |
|---|---|
| Bronchopulmonary dysplasia | 20 (76 %) |
| Congenital high airway obstruction | 2 (8 %) |
| Chromosomal abnormality | 2 (8 %) |
| Laryngomalacia | 1 (4 %) |
| Diaphragm paralysis | 1 (4 %) |

The diagnoses of the patients are listed in Table II. The hospitalization period was 89 ± 31 days. Twenty-two (85%) patients were discharged; however, 4 (15%) patients died.

The mean opening postnatal time for tracheostomy was 54 ± 24 days, and the postmenstrual age (PMA) was 36 ± 3 weeks. The mean time to closure of tracheostomy in the newborns was 387 ± 164 days.

However, when we evaluated only patients with bronchopulmonary dysplasia, the mean opening postnatal time for tracheostomy and the postmenstrual age was 63 ± 16 days and 35 ± 3 weeks, respectively. Moreover, the mean time of closure of tracheostomy in the newborns with tracheostomy was found to be 382 ± 159 days.

While the mean time to open tracheostomy was 63±16 days in patients with BPD, the median was 24.5 (0- 63) days in patients without BPD (p=0.03).

The duration of accidental decannulation developed as an early complication in 8 patients was mean 11 ± 8 days. Aspiration pneumonia in 2, subglottic stenosis in 5, accidental decannulation in 2, tracheocutaneous fistula in 8, suprastomal collapse in 7 and granulation tissue in 2 patients were found to be late complications, which occurred within 90 (IQR: 51- 345) days. Laryngotracheal reconstruction (LTR) was required in 4 patients in the late period after grade 3 subglottic stenosis.

## Discussion

This present study provides information about 26 newborns with tracheostomy for a duration of five years in our NICU. We aimed to find the appropriate time for tracheostomy by examining opening and closing times, complications and demographic characteristics of the patients who were hospitalized and underwent a tracheostomy in our NICU.

Tracheostomy has become a routine clinical intervention in adult critical care, performed in 10–24% of ventilated adult patients.[12] Tracheostomy placement in adults occurred at a median of 9 days (interquartile range 5–14 d) after ICU admission.[12] Improved neonatal intensive care with increased survival of premature infants has changed the management of the neonatal airway. The most important indication of tracheostomy is long-term adherence to mechanical ventilators and the other indication was upper airway obstruction.[13] One of the pediatric tracheostomy studies in which 95 patients with mean age of 5.2 years were involved, revealed non-anatomical [preemie and chronic respiratory diseases (BPD)] and anatomical (vocal cord paralysis, airway stenosis, and syndromic) indications of tracheostomy.[14]

A retrospective analysis of 917 children who underwent tracheotomy from 36 children's hospitals found that the median age of tracheotomy was 0.5 years (inter-quartile range: 0.2–6.3 years). All patients under eighteen years were enrolled in the study in question.[15] In our study, however, we enrolled infants who were admitted to our NICU. Their diagnosis for tracheostomy was similar to our study group.

While most of the published studies on tracheostomy were conducted in the pediatric period, we especially evaluated newborns and infants hospitalized in the infant period.

The timings for the placement of tracheostomy are not specific across many centers.[16,17] In the Upadhyay et al.'s study[17], 41 infants required tracheostomy due to prolonged mechanical ventilator because of BPD. Their median age of tracheostomy placement was 168 days (108–197 IQR), and median PMA was 48 weeks (40–56

IQR). On the other hand, in Mandy et al.'s study, the mean age of tracheostomy placement was 177± 74 days and the PMA at receipt of tracheostomy was 51±10 weeks.[18] In the present study, the mean opening time for tracheostomy was 54 ± 24 days, and the postmenstrual age was 36 ± 3 weeks. Tracheostomy opening time was found later in babies with severe BPD compared to babies with congenital anomalies. However, in this study, it was determined that tracheostomy was performed earlier than the literature.

A multicenter, retrospective study conducted in the year 2015 suggested that babies do better neurodevelopmentally, with early tracheostomy opening. However, the study did not show evidence of an overall attenuation of poor neurodevelopmental outcomes.[19] Furthermore, Luo's[6] retrospective study (n = 72) found a positive significant improvement in linear growth, weight gain, and head circumference in severe BPD patients after tracheostomy. In addition, earlier tracheostomy may give a chance for physical and occupational therapies to start earlier as well as speech/language therapy.

There are no studies with a published evidence-based decanulation algorithm that is for tracheostomy. The soonest indicator of readiness for decanulation is decreased reliance on mechanical ventilation. The most recent clinical consensus statement from the American Academy of Otolaryngology-Head and Neck Surgery discusses the key essentials in readiness for decanulation. Evaluation should involve rigid bronchoscopy and flexible laryngoscopy to ensure airway patency.[20] Our preference is that the child should not have aspiration-type dysphagia and should be independent of the ventilator for the previous 2-4 months. Especially after the age of 2, there should be no need for a ventilator both day and night. This report highlights individualized medical decision making.[10] A study about tracheostomy in which researchers have 12-year experience in the topic in question revealed that median time of decannulation exceeded 2 years.[21] In our study we also used laryngo-bronchoscopy before decannulation, and the mean time of closure of tracheostomy in newborns was 387 ± 164 days (approximately 13 months). In contrast to the other studies, our study revealed that tracheostomy is opened and closed earlier in prolonged intubation. The reason may be that subglottic stenosis and the other complications occur less frequently. In this study, we found that we opened the tracheostomy early without subglottic stenosis. Its closure is also early, but one of the effective aspects is the elimination of the systemic problems that cause the tracheostomy to be opened. As the lung development of the patients accelerates, the tracheostomy closure time is shortened.

Tracheostomy complications are shown in the adult patients more than pediatric patients. Some reports suggest that 39% of preterm children with BPD develop tracheostomy complications.[20,22]

The complications comprise in accidental decannulation, tube plugging, tracheal stenosis, tracheocutaneous fistula, false passage creation, stomal cellulitis, suprastomal collapse and stomal granuloma or keloid formation. Suture abscess, pneumomediastinum, pneumothorax and innominate artery erosion have also been documented less frequently.[9,23] Accidental decannulation in 8 (31%) patients was the early complication that we came across. On the other hand, aspiration pneumonia in 2 (%8), subglottic stenosis in 5 (19%), accidental decannulation in 2 (8%), suprastomal collapse in 7 (27%), tracheocutaneous fistula in 8 (31%) and granulation tissue in 2 (8%) patients were among the late complications we encountered. We haven't seen the other complications like tube plugging, false passage creation, air leaks, artery erosion, and stomal cellulitis. The reason why we do not see the false passage in particular is that we put a maturation suture during the tracheostomy. In addition to this, good nursing care during intensive care follow-up and a scheduled cannula change are also effective.

Also, it was observed in this study that infants did not remain hypoxic with accidental decannulation.

Airway damage and stenosis may occur secondary to chronic trauma of the intubation cannula as well as mechanical ventilator-related lung injury in infants who have not yet completed their lung and airway development, and surgical operation (LTR, balloon dilatation) may be required in severe cases. Therefore, we think that an earlier tracheostomy decision may reduce the risk of airway damage in premature babies with increased susceptibility to injury. However, there is a need for prospective observational studies in which the risk of intubation trauma in the airway is directly observed and evaluated by bronchoscopy, and concrete criteria are developed in this regard. Although many late complications are not seen because the tracheostomy is opened early, suprastomal collapse can be seen in early tracheostomies. This was detected in the long-term follow-up in seven patients due to early tracheostomy.

One of the most important strengths of the study was that it presented the data obtained as a result of a 5-year observation in the NICU. Another strong point is that babies who were discharged with tracheostomy in the early period also had less complications developing after discharge.

The most important limitation of this study was that we did not evaluate polysomnography (PSG) meantime decannulation. PSG can be used prior to decannulation. While PSG does not significantly impact decannulation success rates, it is helpful for determining the presence or absence of central sleep apnea.[24,25]

The limited number of patients included is also a limitation. Instead, a large number of patients should have been involved to evaluate complications.

In conclusion, there is no optimal timing and context for the placement of tracheostomy in infants. A reasonable approach which can be suggested by this report is that chronically ventilated infants should be assessed at approximately 2 months. If there is no evidence that breathing has improved and the patient is still using a mechanical ventilator at high pressures and high oxygen concentration, a tracheostomy placement should be considered within two months. But airway damage and stenosis may occur secondary to chronic trauma of the intubation cannula as well as mechanical ventilator-related lung injury in infants who have not yet completed their lung and airway development, and surgical operation (tracheoplasty, balloon dilatation) may be required in severe cases. Therefore, we think that an earlier tracheostomy decision may reduce the risk of airway damage in premature babies with increased susceptibility to injury. On the other hand, the data obtained from this study can be used in future studies to compare the lung prognosis of infants with early and late tracheostomy.

## Ethical approval

Hacettepe University Non-Interventional Ethics Committee decision number: 2020/11- 28.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: UAT, HTC; data collection: OD, UAT; analysis and interpretation of results: UAT, HTC; draft manuscript preparation: UAT, SY, HTC, ROG. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

# Incidence and risk factors of transient hypothyroxinemia of prematurity: a prospective cohort study

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## ABSTRACT

**Background.** Transient hypothyroxinemia of prematurity (THOP) is characterized by low thyroxine (T4) levels with normal thyroid-stimulating hormone (TSH) levels. This study aimed to determine the incidence and factors associated with THOP.

**Methods.** This prospective cohort study included neonates who were born before 37 weeks of gestation in the neonatal intensive care unit (NICU) between April 2017 and December 2020. Serum TSH and free thyroxine (FT4) levels were routinely screened at 3–5 days and 2, 4, and 6–8 weeks postnatally. The criteria for diagnosis of THOP were a TSH level <7 mU/L with a FT4 level <0.8 ng/dL at any screening timepoint.

**Results.** The incidence of THOP in infants born before 28, 34, and 37 weeks of gestation was 39.5 (17/43), 8.4% (29/343), and 4.8% (35/722), respectively. A multivariate analysis revealed that a gestational age of <28 weeks (adjusted odds ratio [aOR]: 5.35, 95% confidence interval [CI]: 1.89–15.13, p=0.002); 5-min Apgar score of ≤3 (aOR: 5.72, 95% CI: 2.2–14.89, p<0.001); and treatment with aminophylline (aOR: 2.95, 95% CI: 1.08–8.11, p=0.037), dobutamine (aOR: 4.12, 95% CI: 1.55–10.98, p=0.004), or morphine (aOR: 4.91, 95% CI: 1.29–18.74, p=0.011) were associated with an increased risk of THOP. The TSH and FT4 levels in infants with THOP returned to normal ranges by 2 weeks of age.

**Conclusions.** THOP is frequently found in preterm infants. An extremely low gestational age, a low Apgar score, and the use of certain medications in the NICU are risk factors for the development of THOP. Therefore, a thyroid screening program should be implemented for evaluating congenital hypothyroidism (CH) and THOP in preterm neonates in all settings.

**Key words:** neonatal screening, premature birth, risk factors, thyroxine, thyroid-stimulating hormone.

Thyroid hormone is essential for the maturation of many fetal tissues, including the brain, lungs, heart, and skeletal tissues.[1] In preterm infants, thyrotropin or thyroid-stimulating hormone (TSH) levels surge soon after birth, and serum thyroxine (T4) levels are frequently low at 1–2 weeks after birth.[2,3] Thyroid function is affected by several factors, such as immaturity of the hypothalamic-pituitary-thyroid axis, immaturity of thyroid hormone metabolism, loss of maternal T4 supply, iodine imbalance, and neonatal illness.[1,3,4] Thyroid dysfunction, especially low T4 levels, frequently occurs in premature infants and is a potential risk factor for impaired neurodevelopmental outcomes.[5,6] Transient hypothyroxinemia of prematurity (THOP), characterized by low circulating thyroid hormone levels with normal TSH levels, is a common form of thyroid dysfunction, especially in preterm infants born before 30 weeks of gestation. The etiology of THOP is multifactorial, with several contributing factors.[7-9]

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Currently, there is no consensus on the thyroid hormone levels consistent with THOP. The incidence of THOP varies across studies depending on the defined free thyroxine (FT4) and TSH levels. Previous studies have reported that 8–20% of infants with a birth weight <1500 g or a gestational age <34 weeks develop THOP.[10,11]

Neonatal thyroid screening for congenital hypothyroidism (CH) is essential for early detection and prompt treatment of CH. The 2020-2021 European guidelines recommend measuring TSH to detect primary CH and including the measurement of total T4 or FT4 to screen for central CH.[12] In our institute, approximately 3000-3300 births occur per year, of which 210–240 are preterm births that require intensive care. The thyroid screening program for preterm infants born before 37 weeks of gestation was implemented in 2010 to detect abnormal thyroid hormone levels in the neonatal intensive care unit (NICU). According to a previous study, the overall incidence of CH has increased over the past decade, along with an increase in the prevalence of CH in preterm infants owing to increased thyroid screening.[13] Here, we aimed to evaluate the incidence of THOP and identify factors associated with THOP in preterm infants born before 37 weeks of gestation.

## Material and Methods

### *Study population*

This prospective observational study was conducted from April 1, 2017, to December 31, 2020. We enrolled all preterm infants born before 37 weeks of gestation in the NICU. The exclusion criteria were a lack of thyroid screening data, major congenital anomalies, and death before thyroid function tests. Preterm infants with normal thyroid function test results were assigned to the control group and compared with infants diagnosed with THOP. Written informed consent was obtained from the parents of all the included infants.

### *Neonatal TSH screening program*

In our NICU, thyroid screening for preterm neonates was implemented in 2010, and the protocol was revised in 2017. Serial thyroid function tests measuring both serum TSH and FT4 levels are performed routinely at 3–5 days and 2, 4, and 6–8 weeks postnatally in all preterm infants born before 37 weeks of gestation. In preterm infants with hypotension requiring inotropic drugs, thyroid screening is postponed until the drugs have been discontinued for 48 h.

The diagnostic criteria for THOP were a FT4 level <0.8 ng/dL and a TSH level <7 mU/mL at any screening timepoint, which resolved in a subsequent thyroid function test.[14] The diagnostic criteria for CH were either TSH ≥20 mU/L with any FT4 level or TSH >10 mU/L and FT4 <1.00 ng/dL at the time of the first or second screening. The third FT4 and TSH tests were performed in the next 2 weeks. If the FT4 remained <1.00 ng/dL or TSH ≥6 mU/L, the infant was treated with thyroxine 10–15 μg/kg/day.[15,16] The thyroid function tests were conducted 2–4 weeks after starting levothyroxine treatment. Neonatal hyperthyrotropinemia was defined as elevated TSH levels (10–20 mIU/L) with normal FT4 levels (>1.00 ng/dL), with TSH levels normalizing when measured at 28 days of life.

TSH and FT4 levels were measured by electrochemiluminescence immunoassay (ECLIA) using a Modular Analytics E170 machine (Roche Diagnostics, Mannheim, Germany) with an intra-assay coefficient of variation of 1.6–5.0% and inter-assay coefficient of variation of 1.7–5.8%.[13]

### *Data collection*

Demographic data of preterm infants included gestational age, birth weight, Apgar score, ventilator days, and length of

hospital stay. Maternal and obstetric data included the presence of maternal thyroid disease, pregnancy-induced hypertension (PIH), or chorioamnionitis; and antenatal glucocorticoid administration. The gestational age of infants was calculated from menstrual history, ultrasound examination during the first trimester, and the Ballard score test. Neonatal morbidity data were also collected, including respiratory distress syndrome (RDS), transient tachypnoea of the newborn (TTN), patent ductus arteriosus (PDA), hypotension, necrotizing enterocolitis, intraventricular hemorrhage (IVH), and bronchopulmonary dysplasia (BPD). We obtained information on the treatment during admission, including dopamine, dobutamine, morphine, aminophylline, and caffeine administration. In our hospital, aminophylline (intravenous form) is prescribed for preterm infants weighing <1500 g at birth and weaned from mechanical ventilation and for the treatment of apnea of prematurity. BPD was diagnosed based on the National Institute of Child Health and Human Development criteria.[17] Cranial ultrasonography was performed in preterm infants at the first and fourth weeks and at a postmenstrual age of 36 weeks by the pediatric radiologist, and the results were classified into four grades of severity.[18] Hearing screening test was performed using the otoacoustic emission technique or automated auditory brainstem response test (Sentiero, PATH medical GmbH, Germering, Germany) at the time of discharge.

### *Statistical analysis*

The Epicalc package in R Software version 4.4.1 (R Foundation for Statistical Computing, Vienna, Austria) was used for statistical analysis. The Shapiro-Wilk normality test was used to determine whether the sample values were normally distributed. Nominal variables are expressed as the number of infants and percentage. Descriptive statistics for continuous variables are presented as mean ± standard deviation (SD) and median (interquartile range; IQR). For comparisons between the THOP and control group, statistical analysis was performed using Fisher's exact test and chi-square test for categorical variables and Student's t-test and Wilcoxon rank sum test for continuous variables. We performed univariate and multivariate analyses to assess the factors associated with THOP. Independent variables with $p < 0.2$ in the univariate analysis were entered into backward stepwise logistic regression models in the multivariate analysis. Adjusted odds ratios (aORs) and 95% confidence intervals (CIs) were computed for significant variables independently associated with THOP. We applied propensity score methodology to matched subgroups of neonates with similar gestational age and birth weight for evaluating neonatal outcomes. For each infant with THOP, one control infant was randomly selected from the pool of neonates who met the matching criteria. Statistical significance was set at $p < 0.05$.

## Results

During the study period, 1360 infants were hospitalized in the NICU and 781/1360 (58.8%) were inborn and premature. Fifty-nine neonates were excluded due to major congenital anomalies (n=2), death before thyroid function tests (n=11), and no thyroid screening data (n=46). In total, 722 preterm infants were enrolled, of whom 35 (4.8%), 21 (3.0%), and 72 (9.8%) were diagnosed with THOP, CH, and neonatal hyperthyrotropinemia, respectively.

594 infants had normal thyroid function test results (control group). The flow chart of the study population is presented in Fig. 1. The median gestational age in all patients was 33 (31–35) weeks and the overall mortality rate was 2.5% (16/629).

In the THOP group, gestational age in 48.6% (17/35), 20% (7/35), 14.3% (5/35), and 17.1% (6/35) of infants was 24–27, 28–30, 31–33, and

34–36 weeks, respectively. The incidence of THOP in preterm infants born before 28, 34, and 37 weeks of gestation was 39.5% (17/43), 8.4% (29/343), and 4.8% (35/722), respectively. The median age of infants with THOP was 3.8 (3.1–5.5) days. The mean TSH and FT4 levels at the time of diagnosis were 1.87±1.58 mU/L and 0.63±0.18 ng/dL, respectively. The mean FT4 level in infants with THOP gradually increased with time, as shown by the levels of 0.73±0.32, 1.16±0.58, 1.11±0.34, and 1.23±0.44 ng/dL at the first, second, third, and fourth screenings, respectively. The mean TSH levels were 1.99±2.09, 4.9±5.3, 4.4±3.6, and 4.5±2.0 mU/L at the first, second, third, and fourth screenings, respectively. TSH and FT4 levels in infants with THOP normalized within 2 weeks of life (Fig. 2). No infants with THOP received thyroid hormone supplementation.

Table I presents the clinical characteristics between the THOP and control groups. There were no significant differences in sex distribution, delivery mode, antenatal corticosteroid administration, maternal thyroid disease, and PIH between the groups. There

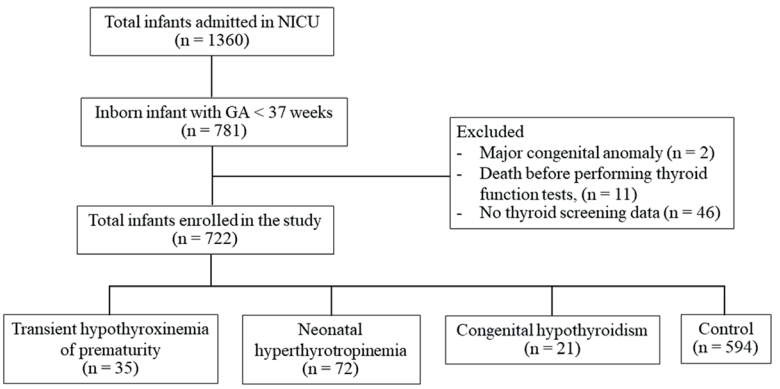


**Fig. 1.** Flow chart of the study population
Abbreviations: GA, gestational age; NICU, neonatal intensive care unit

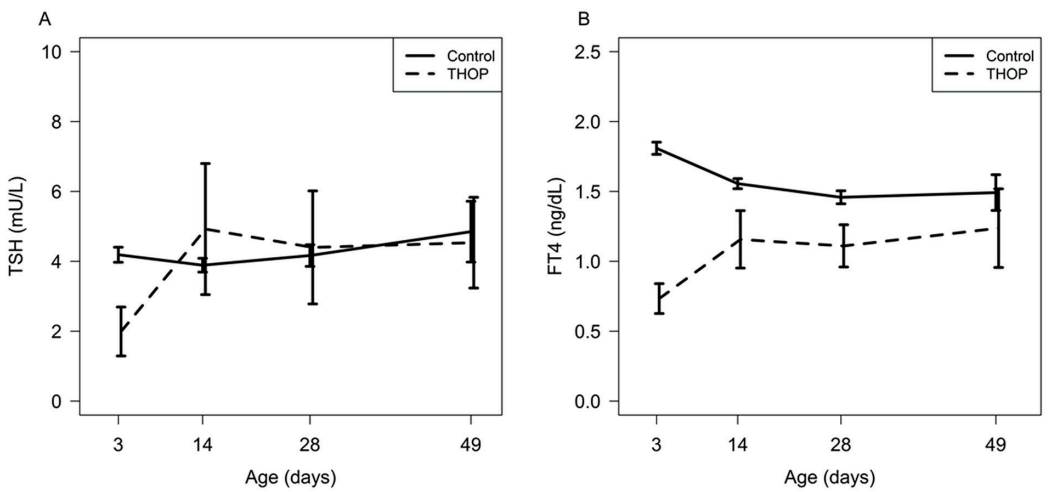


**Fig. 2.** Comparison of the TSH and FT4 levels between the THOP and control groups.
Abbreviations: FT4, free thyroxine; THOP, transient hypothyroxinemia of prematurity; TSH, thyroid-stimulating hormone

**Table I.** Clinical characteristics between the THOP and control groups.

| Variable | THOP (n=35) | Control (n=594) | p |
|---|---|---|---|
| Gestational age, weeks | 28 (25–31.5) | 33 (31–35) | <0.001 |
| 24–27 weeks | 17 (48.6) | 26 (4.4) | <0.001 |
| Birth weight, g | 935 (687–1710) | 1895 (1515–2319) | <0.001 |
| <1000 g | 20 (57.1) | 34 (5.7) | <0.001 |
| 1-min Apgar score | 3 (2–8) | 8 (6–8) | <0.001 |
| 5-min Apgar score | 5 (2–9) | 9 (8–9) | <0.001 |
| Cesarean section | 28 (80) | 466 (78.5) | 0.763 |
| Chorioamnionitis | 3 (8.6) | 9 (1.5) | 0.051 |
| RDS | 28 (80) | 274 (46) | <0.001 |
| TTN | 2 (5.7) | 121 (20.4) | 0.057 |
| PDA | 21 (60) | 104 (17.5) | <0.001 |
| Proven sepsis | 8 (22.9) | 37 (6.2) | 0.002 |
| Hypotension | 26 (74.3) | 148 (24.9) | <0.001 |
| Aminophylline | 25 (71.4) | 134 (22.6) | <0.001 |
| Morphine | 32 (91.4) | 214 (36) | <0.001 |
| Dopamine | 28 (80) | 139 (23.4) | <0.001 |
| Dobutamine | 14 (40) | 60 (10.1) | <0.001 |
| Mechanical ventilation | 33 (94.3) | 255 (42.9) | <0.001 |

Data are expressed as n (%) or median (IQR).
PDA: patent ductus arteriosus, RDS: respiratory distress syndrome, THOP: transient hypothyroxinemia of prematurity, TTN: transient tachypnea of the newborn.

were significant differences in the number of infants weighing <1000 g (57.1% vs. 5.7%, p <0.001) between the groups. The 1-min Apgar score of <4 was found in 18/35 (51.4%) and 55/594 (9.3%) of the infants in the THOP and control groups, respectively, which was a significant difference (p <0.001). Moreover, 15/35 (42.9%) and 21/594 (3.5%) infants had a 5-min Apgar score of <4 in the THOP and control groups, respectively, which was also a significant difference (p <0.001). We found significant differences in the RDS, PDA, hypotension, and proven sepsis rates between the groups. In addition, there were significant differences in the medications used, including aminophylline, caffeine, morphine, dopamine, and dobutamine, between the groups.

The results of univariate and multivariate logistic regression analyses of the risk factors between the groups are presented in Table II. Multivariate regression analysis confirmed that gestational age <28 weeks; 5-min Apgar score <3; and dobutamine, aminophylline, and morphine infusion were found to be independently associated with THOP.

The neonatal outcomes, are presented in Table III. The BPD rate, the rate of mechanical ventilator usage, ventilator days, length of hospital stay, and mortality in the THOP group were significantly higher than those in the control group. Cranial ultrasound was performed in 291/343 (84.8%) infants who were born before 34 weeks of gestation or weighed <1500 g. There were no significant differences in the IVH or PVL rates between the groups. However, when we used the propensity scores to match subgroups of neonates with similar gestational age and birth weight, we found that only the rate of mechanical ventilator usage in infants with THOP was significantly higher than that in the control group. Hearing screening was performed in 86.6% (545/629) of the total infants. In the THOP group, 15 infants did not have hearing screening data because they were referred back to a local hospital (n=8) or due to death (n=7).

**Table II.** Factors associated with THOP development based on univariate and multivariate analyses.

| Factors | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|
| | Crude OR (95% CI) | p | aOR (95% CI) | p |
| GA <28 weeks | 20.63 (9.55–44.59) | <0.001 | 5.35 (1.89–15.13) | 0.002 |
| BW <1000 g | 19.22 (5.37–68.74) | <0.001 | - | |
| 1-min Apgar score ≤3 | 10.38 (5.06–21.29) | <0.001 | - | |
| 5-min Apgar score ≤3 | 20.46 (9.21–45.48) | <0.001 | 5.72 (2.2–14.89) | <0.001 |
| PDA | 7.07 (3.48–14.35) | <0.001 | - | |
| RDS | 4.67 (2.01–10.86) | <0.001 | - | |
| Mechanical ventilation | 21.94 (5.22–92.22) | <0.001 | - | |
| Proven sepsis | 4.46 (1.89–10.5) | 0.002 | - | |
| Dopamine | 13.09 (5.6–30.63) | <0.001 | - | |
| Dobutamine | 5.93 (2.87–12.28) | <0.001 | 4.12 (1.55–10.98) | 0.004 |
| Aminophylline | 8.58 (4.02–18.32) | <0.001 | 2.95 (1.08–8.11) | 0.037 |
| Caffeine | 5.22 (2.6–10.49) | <0.001 | - | |
| Morphine | 18.94 (5.73–62.58) | <0.001 | 4.91 (1.29–18.74) | 0.011 |

aOR (95% CI), adjusted odds ratio (95% confidence interval); BW: birth weight, GA: gestational age, OR: odds ratio, PDA: patent ductus arteriosus, RDS: respiratory distress syndrome, THOP: transient hypothyroxinemia of prematurity.

**Table III.** Neonatal outcomes between the THOP and control groups.

| Variable | Full cohort | | | Match cohort | | |
|---|---|---|---|---|---|---|
| | THOP (n=35) | Control (n=594) | p | THOP (n=35) | Control (n=35) | p |
| BPD, n (%) | 19 (54.3) | 51 (8.6) | <0.001 | 19 (54.3) | 16 (45.7) | 0.633 |
| Cranial U/S, n (%) | 28 (80) | 263 (44.3) | <0.001 | 28 (80) | 24 (68.6) | 0.412 |
| Findings of cranial U/S, n (%) | | | | | | |
| Normal | 10 (35.7) | 106 (40.3) | 0.788 | 10 (35.7) | 10 (41.7) | 0.878 |
| IVH | 16 (57.1) | 151 (57.4) | 1 | 16 (57.1) | 14 (58.3) | 1 |
| PVL | 2 (7.1) | 6 (2.3) | 0.174 | 2 (7.1) | 0 (0) | 0.493 |
| Hearing screening, n (%) | 20 (57.1) | 525 (88.4) | <0.001 | 20 (57.1) | 25 (71.4) | 0.266 |
| Failed screening | 3 (15) | 22 (4.2) | 0.058 | 3 (15) | 1 (4) | 0.309 |
| Mechanical ventilation, n (%) | 33 (94.3) | 255 (42.9) | <0.001 | 33 (94.3) | 25 (71.4) | 0.026 |
| Ventilator use, days | 10 (4–17) | 3 (2–6) | <0.001 | 10 (4–17) | 7 (2–11) | 0.387 |
| LOS, days | 43 (20.5–69.5) | 14 (9–27) | <0.001 | 43 (20.5–69) | 33 (15–52.5) | 0.366 |
| Death, n (%) | 7 (20) | 9 (1.5) | <0.001 | 7 (20) | 3 (8.6) | 0.306 |

## Discussion

In our study, after the revision of the screening program guidelines in 2017 and the use of FT4 and TSH cut-off values of <0.8 ng/dL and <7 mU/mL, respectively, we found an overall incidence of THOP of 39.5%, 8.4%, and 4.8% in preterm infants born before 28, 34, and 37 weeks of gestation, respectively. The incidence of THOP varied across previous studies depending on the cut-off level of thyroxine. Sharma et al.[19] reported an incidence of 19% in infants born before 37 weeks of gestation by using an FT4 level of <0.65 ng/dL, while Kim et al.[20] reported an incidence of 28.9% in infants born before 32 weeks of gestation by using a cut-off FT4 level of <0.7 ng/dL. Further, Yoon et al.[21] reported that

the incidence of THOP was 38.3% in extremely low birth weight infants when using an FT4 level of <0.9 ng/dL. Rabin et al.[10] reported that a low FT4 level (<0.8 ng/dL) was found in 7.9% of infants with a birth weight <1500 g. A study from Scotland used a T4 level less than the 10th percentile for gestational age and reported that 20% of infants born before 34 weeks of gestation developed THOP,[11] while a recent study reported an incidence of 39.2% on performing thyroid function tests between days 10 and 20 postnatally and using FT4 levels less than the reference range.[22]

In our study, the factors associated with THOP were gestational age <28 weeks; 5-min Apgar score ≤3; and aminophylline, dobutamine, and morphine infusion. Similarly, Herring et al.[9] reported that gestational age, dopamine infusion, and mechanical ventilation were associated with THOP. Moreover, male sex, multiple pregnancies, birth weight, small for gestational age, congenital heart disease, and albumin levels were reported as factors associated with THOP in previous studies.[22-24]

Previous studies have revealed an association between low gestational age and low T4 levels.[25] Chung et al. found that infants born before 28 weeks of gestation had lower FT4 and TSH levels through 2 months postnatally than those born after 28 weeks of gestation.[26]

The Apgar scoring system is the standard method for assessing the postnatal clinical status of a newborn; perinatal asphyxia may result in low Apgar scores.[27] During perinatal asphyxia, blood flow to organs apart from the vital organs such as the brain and heart is decreased, resulting in functional abnormalities of the thyroid gland.[28] The effect of asphyxia on thyroid hormone levels has been previously reported. Infants who were delivered via emergency cesarean section with 1-min Apgar scores of <6 had significantly lower T4 and FT4 levels in the cord blood than healthy infants.[29] Similar to our findings, infants with asphyxia, defined as those with Apgar scores ≤3 and ≤5 at 1 and 5 min, respectively, had significantly lower T4, FT4, and triiodothyronine (T3) levels than healthy infants.[30]

Contrary to our findings, critical illnesses, including RDS, sepsis, and PDA, were found to affect thyroid hormone levels in preterm infants.[31-33] In our study, multivariate analysis did not reveal associations between THOP and neonatal illnesses, including RDS, TTN, PDA, and sepsis.

Dopamine and dobutamine are adrenergic neurotransmitters commonly used for inotropic support in preterm infants. Dopamine inhibits TSH secretion through adenylyl cyclase and suppresses T4 secretion and alters hepatic T4 to T3 conversion.[33-35] However, dopamine was not significantly associated with THOP in our study, similar to the findings in a previous study.[33] Dobutamine possibly has the same effect on thyroid function as dopamine, and our study revealed a significant association between THOP and dobutamine infusion.

Morphine and fentanyl exert effects similar to those of opiate drugs that can interfere with serum thyroid hormone transportation.[36] Morphine reduces the TSH, T4, FT4, and T3 levels[32], which is consistent with the association between morphine and THOP found through multivariate analysis in the present study. Aminophylline and caffeine, which are commonly used in preterm infants with recurrent apnea, can cause thyroid dysfunction by increasing the T4, T3, and TSH levels.[34] In our study, aminophylline increased the risk of hypothyroxinemia in preterm infants.

De Felice et al.[37] reported an association between THOP and histological chorioamnionitis. In our study, no significant association was found between THOP and chorioamnionitis (THOP vs. control: 8.6% vs. 1.5%, p=0.051). Maternal preeclampsia can cause a decrease in the placental passage of T4 from mother to infant. A previous study reported that the T4, FT4, free T3, and thyroid binding globulin levels in neonates

born to mothers with maternal preeclampsia were significantly lower than those in neonates born to healthy mothers.[38] However, THOP was not associated with maternal preeclampsia in our study (THOP vs. control: 28.6% vs. 19.9%, p=0.318).

Short-term outcomes in infants with THOP have been previously reported. We found that the rate of mechanical ventilation usage was significantly higher in the THOP group than in the control group, whereas previous studies reported that the rate of BPD and duration of invasive mechanical ventilation usage was significantly higher in the THOP group than in the control group.[22,24] Other neonatal outcomes, including impaired hearing, IVH, and PVL, were not significantly different between the groups, similar to Tan et al.'s findings.[39]

In preterm infants, THOP is characterized by a temporary postnatal reduction in T4 levels with normal TSH levels. T4 and T3 levels continue to increase up to 6–8 weeks postnatally.[4] A previous study reported that FT4 levels gradually increase, normalizing by 7 weeks in infants with hypothyroxinemia.[19] Whereas, we found that all infants with THOP exhibit normal FT4 and TSH levels by 2 weeks postnatally, without thyroxine therapy. These findings suggest that in preterm infants, thyroxine supplementation is not necessary for hypothyroxinemia and that THOP is a physiological phenomenon. Similarly, Uchiyama et al. reported that, in infants with THOP, thyroxine replacement had no beneficial effect on growth and neurodevelopmental outcomes assessed at 3 years.[40] A recent study revealed that THOP is not associated with adverse neurodevelopmental outcomes and does not require thyroxine supplementation.[39] However, monitoring and following up on T4 levels are crucial for confirming the presence of THOP in these infants.

Our study has some notable strengths and limitations. The main strength is that >90% of preterm infants who were delivered in our hospital were enrolled and underwent routine thyroid screening tests; our findings reveal the pattern of FT4 and TSH levels in preterm infants with THOP and healthy preterm infants. The second strength is that we were able to collect complete records of neonatal illnesses and medication use. The main limitation is that the study was performed in a single center with a small sample size. Therefore, large-scale studies are warranted to validate our findings. Moreover, there was a lack of long-term follow-up visits for evaluating growth and neurodevelopmental outcomes in infants with THOP.

In summary, THOP is a form of thyroid dysfunction associated with gestational age in preterm infants. Risk factors for THOP were gestational age <28 weeks; 5-min Apgar score ≤3; and dobutamine, aminophylline, and morphine use. These findings may help optimize care and follow-up of thyroid function in preterm infants. Furthermore, a thyroid screening program should be implemented for evaluating CH and THOP in preterm neonates in all settings.

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**Ethical approval**

The study protocol was approved by the Institutional Review Board and the Ethics Committee of the Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand. (REC 60−033−01−1).

**Author contribution**

The author confirms contribution to the paper as follows: study conception and design: GM, SJ; data collection: GM, MJ; analysis and interpretation of results: GM, MJ, AT; draft

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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**Original Article**

# The prevalence and prognostic effect of hyponatremia in children with COVID-19 pneumonia: a retrospective study

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## ABSTRACT

**Background.** The aim of the study was to examine the effect of hyponatremia at admission as a negative prognostic factor in children hospitalized with COVID-19 pneumonia.

**Methods.** The data of patients aged 1 month-18 years, who were followed with the diagnosis of pneumonia at Çanakkale Onsekiz Mart University Hospital, Department of Pediatrics, between January 2018 and May 2021 were examined, retrospectively. Patients (n=661) were divided into two main groups; COVID-19 pneumonia (n=158) and the other pneumonias [other viral pneumonia (n=161) and pneumonia of unknown etiology (n=342)].

**Results.** Six hundred and twenty-three patients with a median (Q1-Q3) age of 4 (1.5-8) years, 59.4% of whom were male were included in the study. The overall prevalence of hyponatremia at admission was 11.2% and was lower in those with COVID-19 pneumonia than in those with other viral pneumonia (6.4% vs. 15.2%, p=0.013). When evaluated irrespective of their COVID-19 status, hyponatremic patients had a higher supplemental oxygen requirement (OR 2.5 [1.4-4.3], p<0.001), higher need for intensive care unit (ICU) admission (OR 3.7 [1.3–10.2], p=0.009) and longer duration of hospitalization (p=0.016) than the normonatremic patients. In patients with COVID-19 pneumonia, being hyponatremic had no effect on supplemental oxygen requirements or the duration of hospitalization. When hyponatremic patients were evaluated, the supplemental oxygen requirements and duration of hospitalization of those with COVID-19 pneumonia were similar to the other pneumonias (p>0.05 for all comparisons). However, normonatremic COVID-19 pneumonias had higher supplemental oxygen requirements than other viral pneumonias and pneumonia of unknown etiology (OR 4.7 [2.2–10.3], p<0.001; OR 1.6 [1 –2.7], p=0.043, respectively).

**Conclusion.** This study found that hyponatremia at admission is rarer in children with COVID-19 pneumonia than other viral pneumonias and has no effect on supplemental oxygen requirements or the duration of hospitalization.

**Key words:** child, hyponatremia, COVID-19, prevalence, prognosis.

Hyponatremia is the most common electrolyte disturbance in clinical practice and in critically ill children and is present in approximately 3-30% of hospitalized patients. It is also the most common electrolyte disorder in children hospitalized for community-acquired pneumonia (CAP), and its incidence is estimated to be 13-35%.[1,2] However, despite its clinical significance, the prognostic effect of hyponatremia in children with CAP remains unclear.[3]

Hyponatremia in children with pneumonia has been attributed to inappropriate antidiuretic hormone secretion (SIADH) and hypovolemia. Serum sodium levels have been shown to be inversely proportional to inflammatory biomarkers in pneumonia patients/children. Therefore, hyponatremia may be a biomarker for the degree of inflammation and may reflect the severity of infection in CAP.[2] Since the first case reported from Wuhan, China in December

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2019, the coronavirus disease 2019 (COVID-19) pandemic has resulted in approximately 586 million infections and 6.5 million deaths worldwide as of August 2022.[4] It is known that COVID-19 causes hyponatremia in adults because it affects the lungs, heart and kidneys and causes a multisystem inflammatory response.[5,6] COVID-19 may also cause SIADH in children through two potential mechanisms. First, interleukin-6 released from monocytes and macrophages crosses the blood-brain barrier and indirectly stimulates vasopressin release.[2,7] Second, damage to lung tissue and alveolar cells may cause ventilation-perfusion dysfunction and compensatory hypoxic pulmonary vasoconstriction. This, in turn, causes a decrease in atrial tension and an increase in vasopressin release due to insufficient filling of the left atrium. Hyponatremia due to hypovolemia, on the other hand, is due to insufficient oral intake, insensible fluid losses (fever, tachypnea, etc.) and gastrointestinal sodium loss (vomiting, diarrhea, etc.).[2] In addition, hyponatremia and other electrolyte disturbances may develop as SARS-CoV-2 uses angiotensin-converting enzyme 2, which is the main regulator of the renin-angiotensin system, to enter the cell.[8] Therefore, severe inflammation-induced hyponatremia may also be a prognostic marker for negative outcomes in patients infected with COVID-19.[9] Although hyponatremia has been reported[10] in children with multisystem inflammatory syndrome (MIS-C), we could not find a study examining the prevalence and effect on prognosis of hyponatremia in children hospitalized for COVID-19 pneumonia. Therefore, the aim of the study was to determine the prevalence and examine the effect of hyponatremia at admission as a negative prognostic factor in children hospitalized with COVID-19 pneumonia.

## Material and Methods

### *Design sample and data collection*

The data of patients aged 1 month-18 years, who were followed with the diagnosis of CAP at Çanakkale Onsekiz Mart University Hospital, Department of Pediatrics, between January 2018 and May 2021 were examined retrospectively.

Sociodemographic variables as well as clinical features such as symptom duration, presence of dehydration, severity of pneumonia, infectious etiology, supplemental oxygen requirement ($sPO_2$ < %92), need for intensive care unit (ICU) admission (≥24 hours, non-invasive/invasive ventilation), duration of hospitalization (≥24 hours) and laboratory parameters including complete blood count (CBC) parameters [white blood cell (WBC), neutrophil, lymphocyte, eosinophil, monocyte, platelet (PLT) counts and hemoglobin level], C-reactive protein (CRP), procalcitonin (PCT), erythrocyte sedimentation rate (ESR), serum albumin and sodium levels were evaluated from medical records. The patients with marked tachypnea, retraction, grunting respiration, nasal flaring, intermittent apnea, cyanosis, altered mental status, hypoxemia, or capillary refill time ≥2 seconds was classified as having severe pneumonia.

### *Groups and definitions*

The CAP patients were divided into two main groups: the patients with COVID-19 pneumonia (COVID-19 group) and the other pneumonias. The COVID-19 group were further divided into those with a positive nasopharyngeal swab sample SARS-CoV-2 polymerase chain reaction (PCR) test or negative PCR test but having lung tomography compatible with COVID-19 pneumonia by the radiology department. The other pneumonias were also divided into two subgroups consisting of other viral pneumonia (viral group) and pneumonia of unknown etiology (unknown group). The viral group consisted of patients who had nasopharyngeal swab PCR positivity with other respiratory viral agents (influenza, respiratory syncytial virus, etc.). Clinically decided bacterial or viral CAP in which the PCR sample was negative for SARS-CoV-2 or other respiratory viruses or not studied were called "pneumonia of unknown etiology". The patients were divided into three subgroups according to their sodium

levels at admission, as hyponatremic (<135 mmol/L), normonatremic (135-145 mmol/L) and hypernatremic (>145 mmol/L). Patients with primary/secondary immunosuppression, chronic cardio-pulmonary diseases except asthma, hospitalization within 14 days before admission, MIS-C and renal or adrenal insufficiency were excluded from the study.

The study was conducted according to the principles of the Declaration of Helsinki and was approved by the local clinical research ethics committee [dated 06.05.2021, no: 05-26].

### *Outcome measures*

The parameters examined as negative prognostic factors were oxygen requirement, need for ICU admission, and duration of hospitalization and increased and/or decreased levels of inflammatory biomarkers where appropriate in terms of CBC parameters, CRP, PCT, ESR, and albumin and serum sodium level.

### *Statistical analysis*

Descriptive analyses were presented using mean±standard deviation (SD) or median and inter-quartile range (Q1-Q3) for normal or non-normally distributed data, respectively. Frequency (n) and percentage (%) were used for categorical variables. Continuous variables without a normal distribution including demographic variables, presence of dehydration, and severity of pneumonia were compared using the Kruskal-Wallis test and categorical variables using the chi-square test. The study parameters, prevelance of hyponatremia were tested with the chi-square, the Mann-Whitney U, Pearson or Spearman's correlations where appropriate. Odds ratios of the risk factors for adverse outcomes were calculated by binary logistic regression analysis [odds ratio (95% CI)]. A p-value < 0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 23.0 software (IBM, New York, USA).

## Results

### *Patient demographics*

Six hundred and sixty-one patients with CAP were hospitalized between January 2018 and May 2021, 38 of whom were excluded according to the exclusion criteria. Therefore, 623 patients with a median (Q1-Q3) age of 4 (1.5-8) years, 59.4% of whom were male, were included in the study (Fig. 1). The median duration of hospitalization was (Q1-Q3) 4 (2-5), 3 (1-4) and 4 (2-5) days in COVID-19, viral and unknown groups, respectively.

The main clinical features of the groups are shown in Table I. There was a higher rate of vomiting and diarrhea in the COVID-19 group compared to the viral and unknown groups (14.4% vs. 7% and 5%, p=0.002), but there was no difference in the presence of dehydration between these groups (p=0.611). The COVID-19, viral and unknown groups were similar in terms of gender and age (p=0.066, p=0.336, respectively). The viral group had a lower symptom duration than the other two groups at admission (3 vs. 2 days, p=0.001). The rate of severe pneumonia was similar in the COVID-19 and unknown groups (48% vs. 50.6%, respectively, p=0.619) but higher than in the viral group (32.9%, p=0.007).

### *Dysnatremia prevalence*

The overall prevalence of hyponatremia and hypernatremia were 11.2% (n=70) and 2.7% (n=17) at admission in all of the study participants. Although the rates of vomiting/diarrhea and severe pneumonia were higher in the COVID-19 group, they had a lower prevalence of hyponatremia compared to the viral group (6.4% vs. 15.2%, p=0.013).

The main laboratory parameters of the groups are shown in Table II. There was no statistically significant difference between the groups in hyponatremic patients in terms of gender, age, duration of hospitalization, presence of dehydration and severity of pneumonia (p=0.995, p=0.533, p=0.063, p=0.351, p=0.959, respectively).

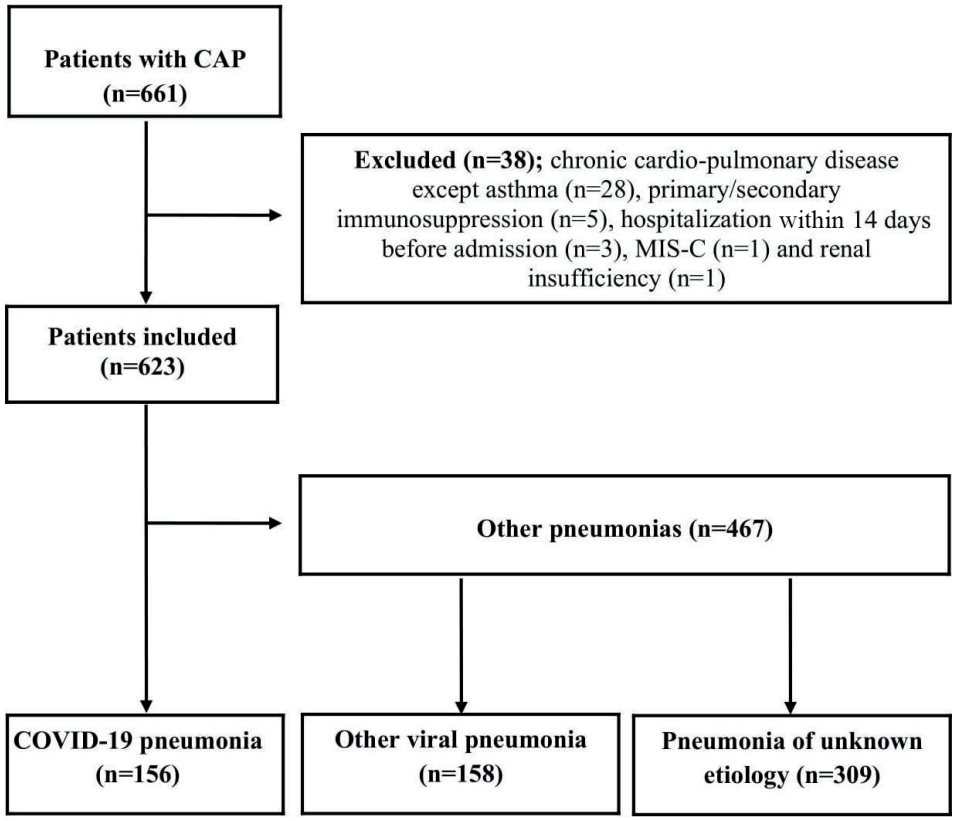


**Fig. 1.** Flow chart of the study. CAP: community acquired pneumonia, MIS-C: multisystem inflammatory syndrome-children.

**Table I.** The basic characteristics of the patients according to their serum sodium levels at admission.

| Basic characteristics | COVID-19 group | | | | Other pneumonia group | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Viral group | | | | Unknown group | | | |
| | Hyponatremic (n = 10) | | Normonatremic (n = 142) | | Hyponatremic (n = 24) | | Normonatremic (n = 131) | | Hyponatremic (n = 36) | | Normonatremic (n = 263) | |
| | N | % | N | % | N | % | N | % | N | % | N | % |
| Gender, male | 6 | 60 | 77 | 54.2 | 14 | 58.3 | 83 | 63.4 | 21 | 58.3 | 159 | 60.5 |
| Age, years | 4.2 (1-6.3) | | 5 (2-9.6) | | 3.2 (1-4.3) | | 4 (1.5-7) | | 3 (1.1-6.3) | | 4 (1.5-8) | |
| Duration of symptoms, day | 3.5 (2-6.2) | | 3 (2-6) | | 2 (1-4.5) | | 2 (1-3) | | 3 (2.2-6) | | 3 (2-5) | |
| Presence of dehydration | 9 | 90 | 13 | 9.2 | 17 | 70.8 | 2 | 1.5 | 24 | 66.7 | 23 | 8.7 |
| Severe pneumonia | 6 | 60 | 67 | 47.2 | 14 | 58.3 | 37 | 28.2 | 20 | 55.6 | 131 | 49.2 |
| Supplemental oxygen requirement | 3 | 30 | 37 | 26.1 | 9 | 37.5 | 9 | 6.9 | 12 | 33.3 | 46 | 17.5 |
| Admission to the ICU | - | - | 3 | 2.1 | 2 | 8.3 | - | - | 4 | 11.1 | 10 | 3.8 |
| Duration of hospitalization, day | 4 (2-5) | | 4 (2-5) | | 4.5 (3-8) | | 3 (1-4) | | 3.5 (2-5) | | 4 (2-5) | |

Numerical data are presented as median (Q1-Q3). ICU: intensive care unit.

**Table II.** Laboratory parameters at admission.

| Laboratory parameters | COVID-19 group | | Other pneumonia group | | | |
|---|---|---|---|---|---|---|
| | | | Viral group | | Unknown group | |
| | Hyponatremic | Normonatremic | Hyponatremic | Normonatremic | Hyponatremic | Normonatremic |
| White blood cell, $10^3$/mm$^3$ | 8.4 (6.5-15.6) | 6.8 (5.1-9.5) | 9.9 (7.4-11.3) | 10.7 (6.9-13.4) | 10.5 (7.4-11.5) | 11.2 (7.6-13.7) |
| Neutrophil, $10^3$/mm$^3$ | 4.4 (2.2-7) | 2.8 (2-4.4) | 5 (2.5-6.6) | 4.2 (2.2-8.4) | 4.4 (2.5-7.3) | 4.7 (2.5-9.3) |
| Lymphocyte, $10^3$/mm$^3$ | 4 (2.1-6.7) | 2.5 (1.6-4.2) | 3.7 (2.1-5.5) | 3.8 (2.3-5) | 3.6 (2.5-5.5) | 3.7 (2.1-5.2) |
| Eosinophil, /mm$^3$ | 100 (0-197) | 85 (0-200) | 100 (0-375) | 80 (0-200) | 100 (0-300) | 70 (0-240) |
| Monocyte, $10^3$/mm$^3$ | 1 (0.6-1.4) | 0.6 (0.4-0.9) | 0.7 (0.6-1.2) | 0.7 (0.4-1) | 0.9 (0.6-1.2) | 0.7 (0.4-1) |
| Hemoglobin, gr/dL | 11.9±0.8 | 12.3±1.3 | 10.9±1.8 | 11.8±1.3 | 11.4±1.8 | 11.9±1.3 |
| Platelets, $10^3$/mm$^3$ | 274 (253-392) | 277 (183-347) | 351 (269-506) | 308 (237-384) | 353 (269-417) | 323 (262-417) |
| CRP, mg/dL | 0.3 (0.1-0.9) | 0.6 (0.2-1.4) | 1.1 (0.7-1.8) | 0.8 (0.3-1.8) | 1.1 (0.3-1.8) | 0.9 (0.1-1.8) |
| PCT, ng/mL | 0.2 (0.1-0.3) | 0.2 (0.1-0.8) | 0.4 (0.1-0.8) | 0.7 (0.1-0.8) | 0.8 (0.2-0.8) | 0.8 (0.1-0.8) |
| ESR, mm/h | 10.8 (7.5-14.2) | 9.7 (4-13) | 9.3 (5-9.7) | 9.7 (7-12) | 10 (6-13.7) | 8 (4-13) |
| Albumin, g/dL | 4.3 (3.7-4.4) | 4.1 (3.6-4.5) | 4 (3.5-4.5) | 4 (3.5-4.6) | 4.2 (3.7-4.5) | 4.2 (3.7-4.6) |
| Sodium, mEq/L | 133 (132.7-134) | 138 (136-139) | 133 (131-134) | 137 (136-138) | 133 (131-134) | 137 (136-139) |

Values are presented as mean ± SD or median (Q1-Q3). CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, PCT: procalcitonin.

### *The association of hyponatremia (at admission) with negative outcomes*

#### *Hyponatremics vs. normonatremics*

When evaluated irrespective of their COVID-19 status, hyponatremic patients had a higher supplemental oxygen requirement (OR 2.5 [1.4-4.3], p<0.001), higher need for intensive care unit (ICU) admission (OR 3.7 [1.3–10.2], p=0.009) and longer duration of hospitalization (p=0.016) than the normonatremic patients. Hyponatremic patients in the viral group also had higher supplemental oxygen requirements (OR 8.1, [2.7–23.6], p<0.001) and longer duration of hospitalization (p<0.001) than their normonatremic counterparts. Furthermore, within the unknown group, hyponatremic patients had higher supplemental oxygen requirements (OR 2.3 [1.1-5), p=0.027), higher need for ICU admission (OR 3.1 [0.9–10.6], p=0.064) than the normonatremic patients. However, supplemental oxygen requirements (p=0.785) and duration of hospitalization (p=0.705) were not different between the hyponatremic and normonatremic patients within the COVID-19 group.

#### *Hyponatremics vs. hyponatremics*

***COVID-19 vs. Viral and COVID-19 vs. Unknown:*** There were no differences among the hyponatremic patients in the COVID-19, the viral and the unknown groups in terms of supplemental oxygen requirements and duration of hospitalization (p>0.05 for all comparisons).

#### *Normonatremics vs. normonatremics*

***COVID-19 vs. Viral:*** Patients in the COVID-19 group had higher supplemental oxygen requirements (OR 4.7 [2.2–10.3], p<0.001) and duration of hospitalization (p<0.001) compared with the viral group.

***COVID-19 vs. Unknown:*** Patients in the COVID-19 group had higher supplemental

**Table III.** The association of baseline laboratory parameters with serum sodium levels.

| Baseline labotatory parameters | General | COVID-19 group | Other pneumonia group | |
|---|---|---|---|---|
| | | | Viral group | Unknown group |
| | r | r | r | r |
| White blood cell† | .046 | -.123 | .200* | .119* |
| Neutrophil† | .112** | -.085 | .250** | .187* |
| Lymphocyte† | -.084* | -.041 | -.076 | -.111* |
| Eosinophil† | .084* | .232** | .034 | .023 |
| Monocyte† | .041 | .012 | .196* | -.008 |
| Hemoglobin‡ | .151*** | .126 | .151* | .147** |
| Platelets† | -.059 | .002 | -.006 | -.079 |
| CRP† | -.071* | -.093 | -.030 | -.040 |
| PCT† | -.016 | -.113 | .068 | .078 |
| ESR† | .048 | .140* | -.030 | .015 |
| Albumin† | .067 | .173* | .039 | .005 |

CRP: C-reactive protein, ESR: erythrocyte sedimentation rate, PCT: procalsitonin. †Spearman's correlation test, ‡Pearson correlation test. *p value<0.05, **p value<0.01, ***p value<0.001.

oxygen requirements (OR 1.6 [1-2.7], p=0.043), but had similar ICU admission (OR 0.5 [0.1–2], p=0.364), and duration of hospitalization (p=0.984) compared with the unknown group.

### *The relationship between inflammatory biomarkers and serum sodium*

When evaluated irrespective of the COVID-19 status, there was a negligible but statistically significant linear correlation between serum sodium levels and lymphocyte count and CRP in a negative direction, and neutrophil , eosinophil count and hemoglobin level in a positive direction (r<0.190, p<0.05). This suggests that those with a higher lymphocyte count and CRP have lower serum sodium levels (Table III).

Patients in the COVID-19 group had lower levels of WBC, neutrophils, lymphocytes, PLT count, CRP and PCT levels than control groups (p<0.05 for all comparisons) (Fig. 2). When hyponatremic patients were evaluated among themselves, those in the COVID-19 group had lower CRP levels than those in the viral group (p=0.015), and lower CRP and PCT levels than those in unknown group (p=0.023, p=0.025, respectively) (Fig. 3).

## Discussion

This study, which included 623 patients, had four main findings. First of all, patients with COVID-19 pneumonia had a lower prevalence of hyponatremia at admission compared to the viral pneumonia group. Furthermore, the hyponatremia of patients with COVID-19 pneumonia had no effect on supplemental oxygen requirements or the duration of hospitalization. In addition, the supplemental oxygen requirement of patients with hyponatremic COVID-19 pneumonia was similar to the that of the hyponatremic control groups. Third, although the supplemental oxygen requirement of normonatremic COVID-19 pneumonia patients was higher than the normonatremic control groups, the supplemental oxygen requirement of patients with hyponatremic COVID-19 pneumonia was similar to the hyponatremic control groups. At last, there was no statistically significant correlation between the biomarkers at admission and hyponatremia, but we found that those with a higher lymphocyte count and CRP had lower serum sodium levels. In addition, patients with hyponatremic COVID-19 pneumonia had lower CRP levels than hyponatremic control groups.

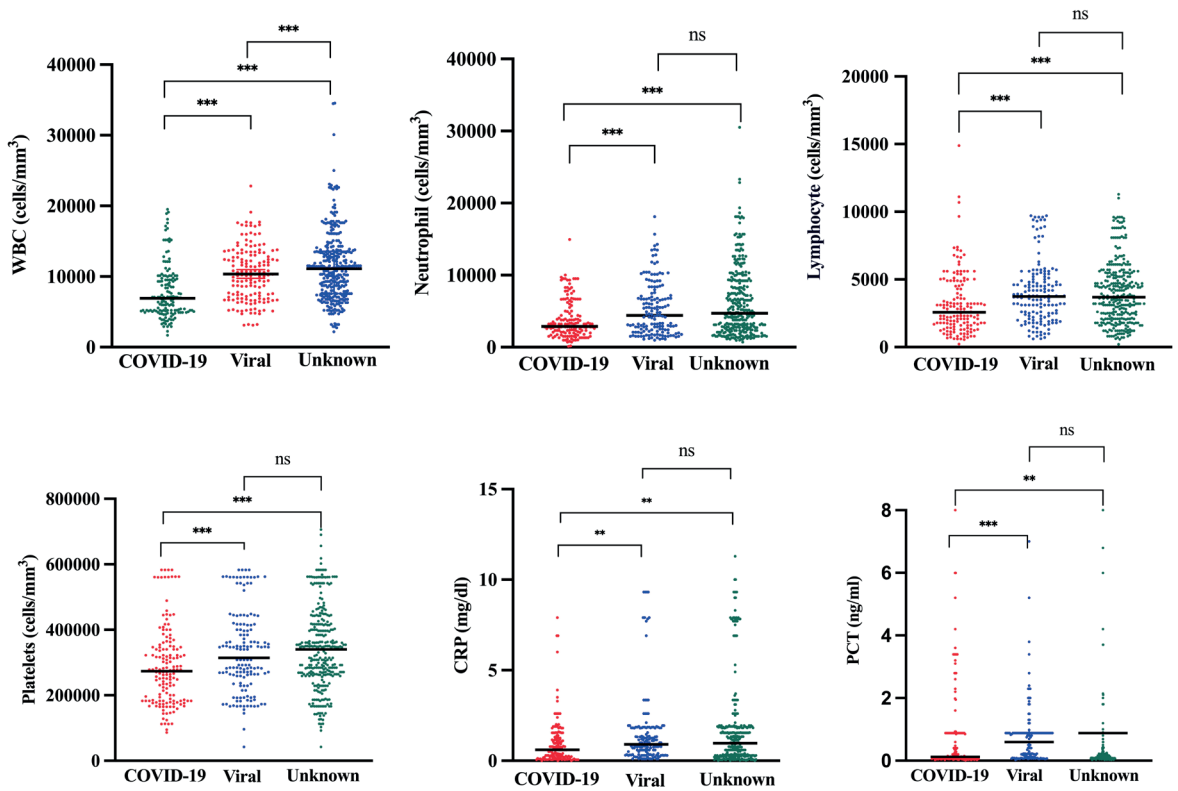


**Fig. 2.** Comparison of WBC, neutrophil, lymphocyte, PLT count, CRP and PCT levels of patients with COVID-19 pneumonia with the control groups (**p value <0.01, ***p value <0.001). CRP: C-reactive protein, PCT: procalcitonin, PLT: platelet, WBC: white blood cell.

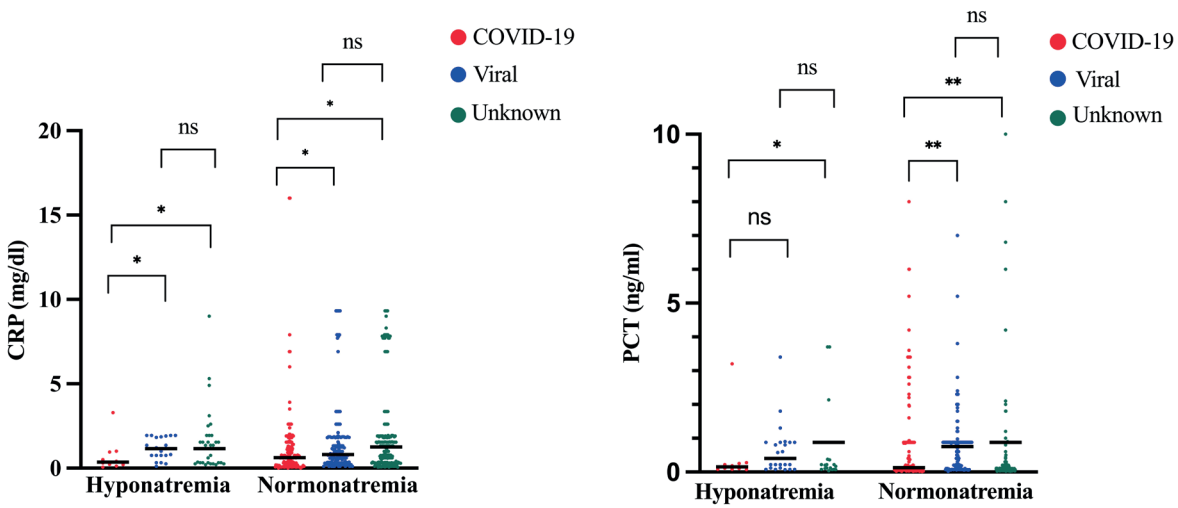


**Fig. 3.** Comparison of CRP and PCT levels of patients with hyponatremic COVID-19 pneumonia with the control groups (*p value <0.05, **p value <0.01). CRP: C-reactive protein, PCT: procalcitonin.

Although CAP is mainly caused by bacteria, viruses are the main etiologic agents in children, and studies report hyponatremia associated with COVID-19 pneumonia.[9,11] Adult studies examining the relationship between COVID-19 and hyponatremia report the prevalence of hyponatremia in COVID-19 as 22.9%-63.6%.[7-9,12,13] In the study where Atila et al.[14]

evaluated 1041 adult patients, the prevalence of hyponatremia was higher at admission in patients with COVID-19 compared to the control group (29.1% vs. 17.6%). In studies conducted in the pre-COVID-19 period in children with lower respiratory tract infections, the prevalence of hyponatremia was reported to be 28.9-80%.[3,15-18] In a study by Park et al.[19] in which they evaluated 3938 children with respiratory tract infections, they reported that the prevalence of hyponatremia was highest (20.7%) in those with human adenovirus (HAdV) infection, and this was due to human adenovirus causing a more intense systemic inflammatory response than other viral infections. In the pediatric population, COVID-19 is known to be an overall less symptomatic and less severe infection compared to HAdV infection. Li et al.[20] reported that children with HAdV infection had higher neutrophil count, CRP, and PCT levels compared to COVID-19. It is also known that while COVID-19 causes a more intense systemic inflammatory response in adults due to the inability to limit the viral spread, it does not cause a significant systemic inflammatory response in children.[21] In our study, the prevalence of general hyponatremia (11.2%) was quite low compared to the literature and was even lower (6.4%) in patients with COVID-19 pneumonia. We speculate that this is due to a lower systemic inflammatory response and thus less SIADH in pediatric COVID-19 pneumonia.

It is thought that hyponatremia may be related to the severity of pneumonia in children.[22] While hyponatremia is considered a poor prognostic factor in adults with COVID-19 pneumonia, little is known about it in children.[3,23] Studies have reported that adults with hyponatremic COVID-19 pneumonia had higher supplemental oxygen requirement, a greater need for ICU admission, a higher mechanical ventilation requirement, a longer duration of hospitalization, and a higher risk of death.[7,9,12-15,24] Studies conducted in the pre-COVID-19 period report that children with hyponatremic lower respiratory tract infections have higher mechanical ventilation requirements, longer ICU stays and longer hospital stays.[3,15] When we evaluated all the patients in our study, those who were hyponatremic had a higher supplemental oxygen requirement, a higher need for ICU admission and a longer duration of hospitalization, consistent with the literature. However, the fact that patients with COVID-19 pneumonia were hyponatremic did not change their supplemental oxygen requirement or duration of hospitalization. This result may be due to the small sample size of patients with hyponatremic COVID-19 pneumonia and further research with a larger sample size is needed to reach a firm conclusion.

Oxygen therapy is necessary in the presence of critical illness in CAP. Long-term oxygen therapy is also required in the treatment of COVID-19, and this period is longer than in influenza cases.[25] In a study by Pucarelli et al.[26], in which they compared children with COVID-19 with other respiratory viral infections, it was reported that those with COVID-19 had a shorter duration of hospitalization but a higher admission to the ICU. Tasar et al.[27] reported that both the admission to the ICU and the duration of hospitalization were higher in children with influenza compared to COVID-19. In our study, the oxygen requirement and duration of hospitalization of patients with hyponatremic COVID-19 pneumonia were similar to those in the hyponatremic control groups. Whereas, patients with normonatremic COVID-19 pneumonia had a higher oxygen requirement and a longer duration of hospitalization than patients in the normonatremic control group, consistent with the literature. We think that this result is due to the small sample size of patients with hyponatremic COVID-19 pneumonia.

Previous studies have shown that hyponatremia is associated with CRP in various infections.[19] Adult studies examining the relationship of hyponatremia with biomarkers in COVID-19 report higher CRP levels, higher WBC counts, and lower lymphocyte counts in hyponatremic patients.[8,9,12] Studies conducted in the pre-COVID-19 period in children report that

hyponatremic children with lower respiratory tract infections have higher WBC counts and CRP levels.[3,15,18] In the study of Liang et al.[28], in which they compared COVID-19 and influenza A infection in children, it was reported that patients with influenza A infection had lower lymphocyte counts and a higher CRP and PCT levels, and lower neutrophil counts in COVID-19. Tasar et al.[27] reported that there was more lymphopenia in the influenza group, but there was no difference between CRP levels. In the study of Liu et al.[29] in which they compared COVID-19 and influenza A/HAdV infection in children, they reported higher leukocyte and neutrophil rates in the influenza A and HAdV group, and higher PRC levels in the COVID-19 group. In our study, we found that patients with hyponatremic COVID-19 pneumonia had lower CRP levels compared to the hyponatremic control groups, consistent with the literature. However, the CBC parameters of patients with hyponatremic COVID-19 pneumonia were not different from the hyponatremic control groups. Evaluation of serum sodium levels in conjunction with inflammatory biomarkers in adult COVID-19 is thought to be helpful both in predicting serious disease and in identifying patients likely to benefit from early interventions.[8] However, our results suggest that assessment of serum sodium levels with inflammatory biomarkers at admission in children cannot yet be used to predict negative outcomes in pediatric COVID-19 pneumonia.

This study has some limitations. First, we could not analyze ICU admissions in all groups due to the small number of patients in some subgroups. Second, we could not exclude viral co-infections since only SARS-CoV-2 PCR was evaluated in the microbiology laboratory during the pandemic period. Finally, we did not evaluate bacterial co-infections in SARS-CoV-2 and other viral PCR positive patients. The strength of this study is that it is one of the first studies with a control group in children with hyponatremic COVID-19 pneumonia and therefore we think that it will add valuable information to the literature.

This study found that hospital admission hyponatremia is a rarer finding in children with COVID-19 pneumonia than other viral pneumonias, and has no effect on supplemental oxygen requirements or the duration of hospitalization. However, when all pneumonia cases were considered, hyponatremic patients had a higher supplemental oxygen requirement, a greater need for ICU admission and a longer duration of hospitalization. Therefore, the authors argue that studies with larger sample sizes are needed to see if serum sodium levels are associated with negative outcomes in children with COVID-19 pneumonia.

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**Ethical approval**

The study was conducted according to the principles of the Declaration of Helsinki and the study was approved by Çanakkale Onsekiz Mart University ethics committee (dated 06.05.2021, no: 05-26).

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: TÇ, DD, ÇFP; data collection: TÇ, DD, ÇFP; analysis and interpretation of results: TÇ, DD, ÇFP; draft manuscript preparation: TÇ, DD, ÇFP. All authors reviewed the results and approved the final version of the manuscript.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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**Original Article**

# Rehospitalization indications of children hospitalized for COVID-19 infections after discharge: Should we suspect long COVID?

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## ABSTRACT

**Background.** Complications that may develop in children after coronavirus disease 2019 (COVID-19) infections are unknown. The "Long COVID" syndrome is a new process that can also be identified in children. Therefore, in this study, the conditions that may develop in children after COVID-19 infection were discussed, and the indications for rehospitalizations were reviewed.

**Methods.** This retrospective cohort study was conducted in a tertiary children's hospital in İzmir, Türkiye. All children who were rehospitalized in the study center after discharge, and the indications for readmissions were screened.

**Results.** Since the beginning of the pandemic, 777 children with COVID-19 infection were hospitalized, including 98 (12.6%) cases rehospitalized for any indication. Fifty-five (56.1%) patients were male, and 43 (43.9%) were female. The mean age of the study population was 79.3±63.5 months (1 month to 17 years). Among these 98 patients, 76 (77.6%) were rehospitalized because of the presence of their primary underlying disease, nonspecific infectious diseases unrelated to COVID-19, and the need to perform certain surgical procedures. The remaining 22 (22.4%) patients presented with symptoms such as fatigue, fever, abdominal pain, and myalgia after the COVID-19 infection. No other underlying cause was detected in approximately one-third of the patients, whose manifestations were found to be consistent with long COVID syndrome.

**Conclusions.** The findings of acute COVID-19 infection are well characterized, but there is still limited data on its long-term outcomes. The majority of the study population that had no underlying disease were thought to have complications from the COVID-19 infection. Therefore, although the incidence rate of long COVID syndrome in childhood has not been revealed so far, it should be kept in mind among relevant differential diagnoses.

**Key words:** Coronavirus disease 2019 (COVID-19), severe acute respiratory syndrome Coronavirus 2 (SARS-CoV-2), long COVID, rehospitalization.

After the resolution of the acute infection of coronavirus disease 2019 (COVID-19) in children, some conditions with synchronous involvement of the cardiac, neurologic, or gastrointestinal systems such as Kawasaki-like syndrome, post-COVID syndrome, or multisystem inflammatory syndrome (MIS-C), may be diagnosed in children.[1] However, there is no precise information about the clinical characteristics of the patients or the indications for hospital readmissions of children with COVID-19.[2]

Data on the rates of and indications for rehospitalizations after COVID-19 infection are mainly retrieved from adult reports, and

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it was emphasized that the leading causes for readmissions were thrombotic processes, reactivation, or reinfection of the COVID-19 disease.[3,4] There are currently limited studies concerning the rehospitalization rates of children excluding those with MIS-C or Kawasaki-like syndrome.[5] In addition, the "long COVID syndrome," which is thought to mainly affect adults, may also be identified in children more frequently than suspected.[6,7] However, to the best of our knowledge, there has been no study focusing on the rehospitalization rates of children who were hospitalized because of COVID-19 infection or the role of long COVID syndrome on readmissions.

Therefore, the objective of this study was to evaluate the rates of and reasons for rehospitalizations of children hospitalized with COVID-19, mainly focusing on patients with symptoms associated with long-term sequelae of the COVID-19 infection without any underlying disease.

## Material and Methods

This retrospective cohort study was conducted in a tertiary children's hospital in İzmir, Türkiye, during the two-year pandemic period from March 11, 2020, to April 1, 2022.

All children previously hospitalized with the diagnosis of acute COVID-19 infection but rehospitalized after their discharge from the hospital were included in the study. In the first week after discharge, the patients had on-call visits, and complaints (if present) were reviewed. The indications for admission of patients requiring rehospitalization after discharge were screened for COVID-19 infection-related complications. Rehospitalizations of the patients due to their underlying primary diseases other than COVID-19-associated conditions were not discussed in the text. Additionally, patients hospitalized for the first time because of MIS-C were excluded from the study. Diagnoses of SARS-CoV-2 were confirmed in all patients by real-time reverse transcription-polymerase chain reaction (RT-PCR) analysis of nasopharyngeal swabs and/or SARS-CoV-2 antibody testing. The decision to discharge the patient was made in each case according to the patient's clinical improvement and current guidelines.[8]

COVID-19 reinfection is defined in the literature as an infection in the same individual within a different period with evidence of genotypic variance, i.e.,post-discharge infection in an individual with two different viral strains within >45 days in highly suspicious cases of COVID-19 or >90 days in asymptomatic cases or cases with low suspicion.[9]

"Long COVID" is a general term used for people who have recovered from COVID-19 but continue to experience complaints such as headaches, fatigue, sleep disturbance, abdominal pain, myalgia or arthralgia, chest tightness, or pain, anosmia or rash for much longer than expected.[10] Regarding its description, various opinions have been expressed about the timeframe of the symptoms, and the Royal College of General Practitioners has defined long COVID as being characterized by "signs and symptoms developed during or following a disease consistent with COVID-19 that have persisted for more than four weeks and whose presence cannot be explained by other alternative diagnoses.[11] During the diagnostic process, additional ultrasonographic, echocardiographic, and, if necessary cranial imaging and laboratory examinations were performed to rule out other possible causes such as infectious causes, MIS-C, malignancy, neurological disorders, and rheumatological diseases.

Indications for readmissions were screened in all patients throughout the two-year study period after the first diagnosis of the COVID-19 infection.

Patients' data were collected from medical records, including demographic and clinical characteristics (age, gender, symptoms, and medical history), underlying diseases, the interval

between discharge and rehospitalization, the reasons for rehospitalizations, and COVID-19 positivity at the time of their rehospitalizations.

### *Statistical analysis*

Statistical analyzes were performed using SPSS Statistical Software (version 22; SPSS, Chicago, IL, U.S.A.). Data with a normal distribution were expressed as mean±standard deviation (mean±SD). In addition, the chi-square test was used to investigate the correlations among variables (if any). The level of statistical significance was set at $p<0.05$.

Ethics approval for this study was obtained from the Institutional Review Board of Dr. Behcet Uz Children's Training and Research Hospital (decision no: 2021/15-08).

## Results

From the beginning of the pandemic until April 2022, 777 children with COVID-19 were hospitalized in the pandemic clinic. Of these, 98 (12.6%) were rehospitalized for any reason. Fifty-five patients were male (56.1%), and 43 were female (43.9%). The mean age of the study population was 79.3±63.5 months (1 month to 17 years).

Among these 98 patients, 76 (77.6%) were rehospitalized because of their primary underlying diseases or a nonspecific infectious disease unrelated to COVID-19 or the need for a certain surgical procedure. Fig. 1 demonstrates the flow chart of the patients included in the study. The most common indication for rehospitalization was the presence of an underlying disease, and the majority of this group consisted of hematology-oncology and bone marrow transplant patients (n:22; 22.4%). Twenty-two (10.2%) patients required palliative treatment, and 7 (7.2%) had a neurological disease. The reasons for hospitalizations and the units they were hospitalized are given in Table I.

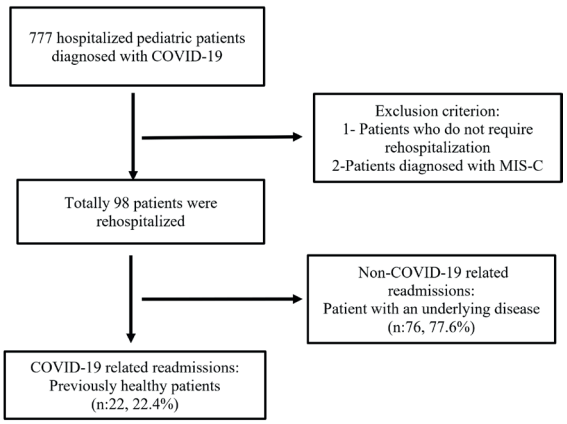


**Fig. 1.** Distribution of the patients included in the study.

**Table I.** Reasons for readmission after discharge of patients diagnosed with COVID-19 infection (n=98).

| Distribution of rehospitalized patients according to the service | n (%) |
|---|---|
| Infectious disease unit | 22 (22.4%) |
| Hematology-oncology unit | 22 (22.4%) |
| Surgical unit | 13 (13.3%) |
| Palliative unit | 10 (10.2%) |
| Neurology unit | 7 (7.2%) |
| Other units | 24 (24.5%) |
| **Number of patients according to hospitalization reasons** | **n (%)** |
| Underlying diseases | 59 (60.2%) |
| COVID-19 related conditions | 22 (22.4%) |
| Surgical reasons | 9 (9.2%) |
| Non-specific infectious diseases | 8 (8.2%) |
| Total | 98 (100%) |

Only 22 (22.4%) patients, including 16 (72.7%) male and 6 (27.3%) female cases, were rehospitalized because of probable post-COVID-19-associated conditions. The mean age of these patients was 98.9±68.4 months (range from 2 months to 17 years). The median time interval between the readmission to the hospital after discharge was 32.5 days (range: 3- 521 days). The most common symptoms of presentation were fatigue, myalgia, and fever. In addition, the following symptoms observed were chest pain, severe abdominal pain, diarrhea, vomiting, headache, cough, and syncope (Table II).

**Table II.** Demographic characteristics and the most common symptoms of patients rehospitalized related to COVID-19 (n=22).

| | |
|---|---|
| Gender, n (%) | |
| Male | 16 (72.7%) |
| Female | 6 (27.3%) |
| Age, months (mean±SD) | 98.9±68.4 |
| The interval between discharge and rehospitalization, days (median; range) | 32.5 days (range 3 to 521 days) |
| Patients with underlying disease, n (%) | 4 (18.2%) |
| Duration of the first hospitalization, days (mean±SD) | 6.9±6.1 |
| Duration of rehospitalization, days (mean±SD) | 7±4.6 |
| Number of patients reinfected with COVID-19 | 3 (13.6%) |
| Most prevalent readmission signs and symptoms, n (%) | |
| Fatigue | 18 (81.8%) |
| Myalgia, joint pain | 10 (45.4%) |
| Fever | 8 (36.4%) |
| Chest pain | 4 (18.2%) |
| Severe abdominal pain | 4 (18.2%) |
| Diarrhea, vomiting | 4 (18.2%) |
| Headache | 2 (9%) |
| Cough | 1 ( 4.5%) |
| Syncope | 1 ( 4.5%) |

n: number, SD: standard deviation

Three of the 22 patients who presented with fever or fatigue with RT-PCR test positivity for the delta variant for the second time were followed up in consideration of COVID-19 reinfection. In addition, an RT-PCR positive male patient diagnosed with acute myeloid leukemia (AML) was rehospitalized 21 days after discharge.

Two patients presented with complaints of headache, myalgia, and fatigue. One of them, a 17-year-old boy without concomitant obesity or underlying disease, presented with sudden unilateral vision loss, severe headache, and syncope three months after the onset of the COVID-19 infection. Dural venous sinus thrombosis was detected in his cranial magnetic resonance imaging (MRI), necessitating subcutaneous enoxaparin treatment. This patient was maintained regularly on warfarin treatment after discharge.

One of the four patients who reported chest pain had a history of syncope, and the examinations revealed vasovagal syncope. At admission, another patient diagnosed with myocarditis had elevated serum troponin-c (TnC) and creatinine kinase-myocardial band (CKMB) that returned to average values during follow-up without specific therapy. In addition, increased serum TnC and CKMB levels were detected in the youngest patient of the study, who had been admitted with a fever. Concurrent control electrocardiograms (ECG) were unremarkable, and patent foramen ovale and suspicious myocarditis were observed in the echocardiography (ECHO) of the patient. Therefore, the patient was monitored for myocarditis for three weeks.

Seven patients presenting with common symptoms such as fever, fatigue, and diarrhea did not meet MIS-C criteria based on their medical history, laboratory findings, and physical examination findings. No other underlying infectious, rheumatological causes, or other reasons were observed in their detailed analysis. In addition, six out of 22 (27.3%)

patients (excluding one case) were readmitted at least three weeks after hospital discharge. While fatigue and myalgia were the main findings in these patients, cough, diarrhea-vomiting, joint pain, and fever were other accompanying findings.

The last four patients presented with severe abdominal pain, accompanied by fatigue or fever, and were diagnosed with acute appendicitis. The diagnosis of appendicitis was confirmed with a histopathology examination. In addition, perforated phlegmonous appendicitis was detected in two of them. Therefore, these patients were referred to the general surgery department after detailed radiological examinations and biochemical tests. The clinical characteristics of hospitalized patients associated with COVID-19 are detailed in Table III.

## Discussion

Variable symptoms have been identified in people after the COVID-19 infection at varying time intervals. However, the most common symptoms of this condition, which have received epidemiologically different definitions, are fatigue, shortness of breath, chest pain, myalgia, and cognitive dysfunction, which

**Table III.** Clinical characteristics and readmission reasons of patients requiring rehospitalization caused by COVID-19-related-conditions.

| Case number | Sex | Age (months) | Underlying diseases | Time from positive to second hospitalization (days) | Presentation symptoms | COVID-19 reinfection |
|---|---|---|---|---|---|---|
| Case 1 | M | 96 | Panic disorder | 8 | Chest pain, fatigue, myalgia | N |
| Case 2 | M | 36 | N | 236 | Fever, fatigue, cough | N |
| Case 3 | F | 24 | N | 32 | Fever, fatigue | N |
| Case 4 | F | 2 | N | 4 | Fever | N |
| Case 5 | M | 144 | N | 16 | Fatigue, severe abdominal pain, myalgia | N |
| Case 6 | M | 156 | N | 38 | Chest pain, fatigue, myalgia | N |
| Case 7 | F | 144 | N | 6 | Chest pain, fatigue, syncope, myalgia | N |
| Case 8 | M | 204 | N | 87 | Headache, fatigue, syncope, myalgia | N |
| Case 9 | M | 156 | Asthma | 521 | Fatigue, myalgia | Y |
| Case 10 | M | 168 | Obesity | 16 | Headache, fatigue, vomiting-diarrhea, myalgia | N |
| Case 11 | F | 96 | N | 125 | Fatigue, diarrhea, myalgia | N |
| Case 12 | M | 60 | N | 447 | Fatigue | Y |
| Case 13 | F | 108 | N | 39 | Fever, fatigue, severe abdominal pain | N |
| Case 14 | M | 2 | N | 3 | Fatigue, vomiting | N |
| Case 15 | F | 120 | N | 10 | Chest pain, fatigue, myalgia | N |
| Case 16 | M | 7 | N | 30 | Fever, fatigue, diarrhea | N |
| Case 17 | M | 204 | N | 33 | Fatigue, myalgia, joint pain | N |
| Case 18 | M | 8 | N | 18 | Fever, diarrhea | N |
| Case 19 | M | 22 | Tay-Sachs | 64 | Fever | Y |
| Case 20 | M | 108 | AML | 21 | Fatigue | N |
| Case 21 | M | 132 | N | 44 | Fatigue, severe abdominal pain | N |
| Case 22 | M | 180 | N | 175 | Fever, severe abdominal pain | N |

F: Female, M: Male, AML: Acute myeloid leukemia, N: None, Y:Yes

cannot be explained otherwise and impact daily life.[12] Although adult studies on this condition are frequently cited in the literature, pediatric studies are rarely mentioned.[2] Therefore, this study screened the indications for rehospitalizations after discharge of the patients due to the COVID-19 infection. In this study, 98 (12.6%) of the 777 patients diagnosed with COVID-19 infection were rehospitalized for different indications. Of these 98 patients, only 22 (22.4%) were hospitalized with a cause associated with COVID-19, and the remaining patients were rehospitalized due to their underlying diseases.

Similar to the literature[10,13], the most common presenting symptoms were fatigue, myalgia, and fever in our report, followed by chest pain, severe abdominal pain, diarrhea, vomiting, headache, cough, and syncope. These symptoms are also seen in other diseases, so it must not be forgotten that the diagnosis of post-COVID or long COVID should be considered a diagnosis of exclusion.

In the current study, COVID-19 reinfection was detected in three patients. While more adult studies[14] on COVID-19 reinfection have been published, studies on children have rarely been performed. While there is a case report[15] of COVID-19 reinfection in three children with underlying cancer in the literature, unlike our study, only one of these three patients had an underlying metabolic disease. In our study, a patient with acute myeloblastic leukemia (AML) was hospitalized again due to fatigue on the 21st day of his SARS-CoV-2 RT-PCR positivity. However, the patient whose SARS-CoV-2 RT-PCR positivity continued was not considered a COVID-19 reinfection. So in this report, the cause of these reinfections was associated with the emergence of new strains rather than underlying conditions.

One patient was hospitalized to investigate the causes of headache and loss of vision, he did not have any organic pathology such as hypertension, obesity, diabetes mellitus, etc., and a dural sinus venous thrombosis was detected in his cranial imaging. Hypercoagulation with vascular endothelial damage and the consequent risk of venous and arterial thrombotic complications are well-known and common findings that may accompany COVID-19 for a long time.[16] Pediatric hematologists have developed guidelines for thromboprophylaxis as cases of thrombosis concurrent with COVID-19 have increased. However, these guidelines were based on extrapolation of adult data and were not supported by data on the incidence of or risk factors for thrombosis in children or adolescents with acute or post-COVID-19.[17] A patient from the literature developed thrombosis 30 days after discharge, similar to our study. This study reported that age ≥12, cancer, central venous catheters, and MIS-C were significantly associated with thrombosis.[18] Similar to our case, he was an adolescent, but differently; he had an underlying cancer disease and a central venous catheter. As another cause of thrombosis, concomitant D-dimer elevation has been reported in patients diagnosed with COVID-19.[19] Unlike these reports, in our case, D-dimer and other coagulation parameters were within normal limits both during COVID-19 positivity and when the dural venous sinus thrombosis was detected. Therefore, no anticoagulant prophylaxis was initiated when the diagnosis of COVID-19 was made because the patient had no underlying risk factors. However, once thrombosis was detected, subcutaneous enoxaparin treatment was initiated, and the patient was maintained on regular warfarin treatment after discharge.

Chest pain and heart failure were also important reasons for readmission within the first 14 days after discharge and in the long term.[20] Four patients presented with chest pain as the primary complaint in our study within the first two weeks of their COVID-19 positivity. In our study, the most common symptom was chest pain detected on cardiac evaluation after acute COVID-19 infection, similar to the relevant literature findings.[21] In addition, similar to our research, ECHO findings were completely normal in the cited study, but differently,

sinus bradycardia was detected on the ECG of a smaller number of patients, and it has been stated in the literature that there is no routine ECG and echo requirement in asymptomatic children.[21] However, another issue that should not be forgotten is that myocarditis results in sudden death among children.[22] Diagnosis and treatment of myocarditis are controversial in pediatric patients, and clinical findings, ECG, elevated troponin levels, cardiac enzymes, and echo are helpful diagnostic tools.[23] During the pandemic, elevations of cardiac enzymes such as TnC and CKMB caused anxiety in patients infected with COVID-19. In the pediatric age, the role of troponin in myocarditis has been much less defined than in adults. However, data on increased troponin I levels as a marker of myocardial injury in children is still accumulating.[24]

Although cardiological involvement in children with COVID-19 is rarely seen, it should be emphasized that all pediatric patients should be evaluated in terms of cardiologic abnormalities.[21] In the example given in our study, a 6-fold increase in cardiac enzymes was detected in an infant who presented with a history of fever, weakness, and vomiting, and he was followed up and treated in favor of myocarditis with additional cardiological evaluation.

Gastrointestinal (GI) symptoms, such as diarrhea and abdominal pain, are prevalent features of the SARS-CoV-2 infection. Moreover, case reports of appendicitis have been reported in the literature in SARS-CoV-2-positive patients.[25,26] Four patients in our report presented with severe abdominal pain and were diagnosed with appendicitis. In the literature, cases of perforated appendicitis accompanying COVID-19 were mainly emphasized, and their presence was attributed to delays in diagnosis.[27]

Similarly, two of the four cases of appendicitis detected in our study were perforated. In addition, a study by Malhotra et al.[28] compared the cases with SARS-CoV-2 infection having a longer duration of symptoms before admission with those SARS-CoV-2 negative patients. According to the study, the presence of intense inflammation raises the risk of rupture. Likewise, it is unknown whether a COVID-19 infection accompanied by an exaggerated inflammatory response is also a potential trigger for appendicitis. Although the triggering mechanism of appendicitis has not been clarified yet, clinicians should be informed that appendicitis may occur in children as an infectious manifestation of the SARS-CoV-2 infection.

Long COVID syndrome is another current issue with the COVID-19 pandemic, and data on pediatric patients has just begun to be presented. Although no universally accepted definition of this syndrome exists, persistent fatigue, shortness of breath, cough, joint pain, chest pain, myalgia, headache, and other symptoms following COVID-19 should be considered if they cannot be attributed to another cause.[7] Studies focusing on long COVID syndrome in children are rare, despite evidence showing that children and young people are also affected by long COVID syndrome.[2] Similar to the literature[29], fatigue, and myalgia were the most prevalent readmission symptoms of long COVID in this report. The median time interval between readmission to the hospital and hospital discharge was 32.5 days, while it was longer in other studies, which were associated with discrepancies in inclusion criteria. Unlike other studies, this report included all readmitted patients and long COVID cases to evaluate post-COVID complications.

Some considerations should be noted when interpreting the results. Firstly, this was a retrospective study with inherent limitations compared to randomized trials. Secondly, only hospitalized patients were evaluated, and outpatients were excluded from the study.

However, it must be emphasized that the current study is the first report characterizing pediatric patients with COVID-19 who were followed up for a long time and readmitted after discharge in case of need.

In our study, patients readmitted with one of the symptoms of COVID-19 after discharge were discussed, and other underlying indications for readmissions were screened. As previously stated, three patients had reinfections, and four had appendicitis. The medical histories of all the remaining COVID-19 patients revealed that their symptoms persisted after their discharge from the hospital. However, all of the cases included in the study, as well as patients with appendicitis, are thought to have complications associated with the COVID-19 infection. In addition, the duration of symptoms, like the definition of long COVID syndrome, is not delineated, and in our study, the course of symptoms was variable. In conclusion, further research on complications that may arise after COVID-19 infection, precise definitions, and durations of long COVID syndrome are required.

### Ethical approval

Ethics approval was obtained from the Institutional Review Board of Dr. Behcet Uz Children's Training and Research Hospital (decision no: 2021/15-08).

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: EC, İD, NB; data collection: EB, EK, MG, AAK, ŞŞ, MYÇ, GGÖ; analysis and interpretation of results: EC, İD; draft manuscript preparation: EC, İD, NB. All authors reviewed the results and approved the final version of the manuscript.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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**Original Article**

# The effects of measures taken during the COVID-19 pandemic on the seasonal dynamics of respiratory viruses in children

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## ABSTRACT

**Background.** We aimed to evaluate the effects of public health measures taken during the COVID-19 pandemic on respiratory viruses.

**Methods.** The study was conducted between February 1, 2021 and December 1, 2022. Patients aged 1 month to 18 years hospitalized for infectious diseases were tested for SARS-CoV-2 and respiratory viruses by multiplex PCR.

**Results.** Of the total 1173 patients, 56.2% were male and 43.8% were female, and 47.5% of the patients were under 24 months of age. The viruses detected were SARS-CoV-2 31.9%, human rhinovirus/enterovirus 19.4%, respiratory syncytial virus (RSV) 9.3%, parainfluenza virus 7%, adenovirus 6%, seasonal coronavirus 5.2%, bocavirus 3.8%, influenza 3.1%, and metapneumovirus 2.8%. Among the patients, 386 were hospitalized with lower respiratory tract infections, 238 with upper respiratory tract infections, 202 to evaluate fever etiology, 111 with acute gastroenteritis and 236 with other diagnoses. Of these patients, 113 were admitted to the intensive care unit. Intensive care unit admission rates were statistically significantly higher for bocavirus and RSV, in those hospitalized between July 1, 2021 and July 1, 2022 (first period when schools were held full-time face-to-face at all grades) and in children aged 1-24 months.

**Conclusions.** Public health measures taken during the COVID-19 pandemic have affected the seasonal distribution of respiratory viruses and the severity of illness in children.

**Key words:** SARS-CoV-2, pandemic, respiratory viruses, children, non-pharmacological interventions.

Viruses, among the leading agents of infectious diseases, are one of the most important causes of respiratory tract infections that can lead to severe morbidity and mortality in childhood.[1] Recognised as respiratory tract viruses, adenovirus (AoV), human bocavirus (HBoV), human coronaviruses (HCoV), human metapneumovirus (HMPV), human parainfluenza 1–4 viruses (HPIV 1–4), human rhinovirus/enterovirus (HRV/EV), influenza virus (IV) A and B, respiratory syncytial virus (RSV), and parechovirus play a predisposing role in the development of acute respiratory tract infections and are widely circulated.[2]

The epidemiology of respiratory viruses in acute respiratory tract infections varies seasonally from region to region around the world.[2,3] The annual virus distribution in the northern hemisphere is as follows: IV and RSV in December-January-February (winter); rhinoviruses in spring and fall; HPIV type 1 in

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winter, type 3 in spring-summer; some non-rhinovirus enteroviruses in summer; HMPV year-round, especially in spring; AoV and HBoV year-round.[2] HCoV infections are primarily detected in the winter and spring months in temperate climates and can be detected at low levels throughout the year. While HCoV OC43 and 229E subtypes are more common in winter and spring, there are reports that NL63 and HKU1 subtypes are more predominantly detected in winter.[3]

COVID-19, caused by the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), the seventh known coronavirus to infect humans, has caused humanity to face a coronavirus pandemic for the first time.[3] Uncertainties about the disease in the early stages of the pandemic, the lack of available treatment or protective vaccines against the virus, and the rapid spread of the infection around the world have created a serious burden on health services.[4]

Serious public health measures have been taken to slow the spread of infection and gain time to increase knowledge about SARS-CoV-2 and the disease caused by this virus. These measures led to changes in people's lifestyles and caused serious disruptions in interpersonal social activities within the community.[4]

In the first year of the COVID-19 pandemic, significant changes were noticed in the circulation of other respiratory viruses.[4] The aim of this study was to evaluate the change in the seasonal dynamics of respiratory viruses and its reflection on the clinic, especially between the first year when the bans were completely lifted and face-to-face education started and the following year.

## Material and Methods

Data from this single-center, cross-sectional study was collected between February 1, 2021 and December 1, 2022 at Prof. Dr. Cemil Taşcıoğlu City Hospital, a tertiary education and research hospital with a capacity of approximately 100 pediatric beds in Istanbul. This hospital is a reference training and research hospital serving approximately 350,000 pediatric outpatients and 3500 pediatric inpatients annually. The study did not include almost the first year of the pandemic because we started evaluating respiratory tract viruses by multiplex PCR in our hospital approximately one year after the pandemic.

All children included in the study were pediatric patients between the ages of 1 month and 18 years who were hospitalized with infectious causes and whose etiology could not be determined. Swabs from the nasopharyngeal region were tested for SARS-CoV-2 and other respiratory viruses by multiplex polymerase chain reaction (PCR).

The ethical committee of Prof. Dr Cemil Tascioglu City Hospital approved the study (No: 2022/315).

Demographic characteristics, hospitalization diagnoses, intensive care requirements of the patients, and months and seasons in which viruses were detected were obtained from hospital medical records.

### *Non-pharmacological pandemic control in the world and in Türkiye[4,5]*

Personal non-pharmacological measures included the use of face masks, respiratory etiquette, and the importance of hand hygiene, maintaining a social distance of at least 2 meters between individuals, screening and isolating sick individuals, identifying and quarantining people in contact.

Non-pharmacological measures taken by the society were making the use of face masks mandatory in public indoor environments and public transportation, closing schools and childcare facilities and switching to online education, postponing indoor meetings and major events, closing non-essential businesses within the scope of stay-at-home and quarantine measures, prohibiting eating indoors in restaurants, tracking contacts, and

informing the public about the routes and dynamics of virus transmission through various communication methods.

The environmental non-pharmacological measure was the disinfection of contacted surfaces.

Non-pharmacological measures taken by countries were travel restrictions between cities and countries, border closures, health screenings at entry and exit points, quarantine measures at entrances, and compulsory screenings.

In our country, almost all of the above-mentioned measures were implemented in the early stages of the pandemic, and curfews were imposed on certain age groups (such as those over 65 and under 20) at certain hours.

### *Statistical analysis*

The relationship between the measures taken in terms of public health in our country and the detected data was analyzed. SPSS 15.0 for Windows program was used for statistical analysis. Descriptive statistics were given as numbers and percentages for categorical variables, and numerical variables were presented as mean, standard deviation, minimum and maximum. The rates in the groups were compared with the chi-square test. The statistical alpha significance level was accepted as $p<0.05$.

## Results

A total of 1173 patients who were hospitalized, followed and treated with prediagnoses of fever etiology, acute gastroenteritis, febrile seizures, encephalitis, sepsis, rash, myocarditis, soft tissue infections, lymphadenopathy or eye infections, and especially acute respiratory tract infections, were tested for SARS-CoV-2 and other respiratory tract viruses by multiplex PCR. 659 patients were male and 514 were female, with a median age of 29.5 months (1-216 months). Five hundred fifty-seven patients were between 1 month and 24 months, 255 patients between 25 and 60 months, 183 patients between 61 and 120 months, and 178 patients were 121 months and older.

Five hundred thirteen viruses were detected in 631 patients tested in 2021 and 526 viruses were detected in 542 patients tested in 2022. The five most frequently detected viruses in 2021 were SARS-CoV-2, HRV/EV, RSV, HPIV (47 patients, 43 of which had type 3 HPIV), and HBoV, while those detected in 2022 were SARS-CoV-2, HRV/EV, AoV, RSV, HCoVs (42 patients, most frequently OC43 in 17 patients). The distribution of detected viruses by months and years is shown in Fig. 1.

In the five-month period between February 1, 2021 and July 1, 2021, when school attendance was online and the restrictions were not entirely lifted, 88 patients were detected to be positive. Of these, 75% (66 patients) were positive for SARS-CoV-2. Among the rest, HRV/EV (12 patients) was the most commonly detected, followed by AoV (7 patients), HCoV OC43 (3 patients), HPIV type 3 and HBoV (2 patients each) and RSV and Parechovirus (1 patient each). Seven hundred fifty-seven viruses were detected in the first one-year period (July 1, 2021- July 2022), when the restrictions were lifted completely and face-to-face education started at all levels of schools. SARS-CoV-2 was detected in 270 patients, followed by HRV/EV (164 patients), RSV (69 patients), AoV (49 patients), HPIV type 3 (47 patients), HBoV (39 patients), influenza A (32 patients), HMPV (30 patients), and HCoV OC43 (21 patients).

In 834 patients, at least one SARS-CoV-2 or other viruses, alone or in combination, were detected. The viruses detected and their numbers are shown in Table I. Among the patients with confirmed HPIV, 51 had type 3, 20 had type 1, 6 had type 4 and 5 had type 2. The subtypes detected in patients with HCoV were OC43 in 33, NL63 in 11, HKU1 in 10, and 229E in 7. The mean age of SARS-CoV-2 positive patients was 64.3 months (1-214 months), and 50.4% were between 1 month-24 months (188 patients) and 22.5% were 121 months and older (84 patients).

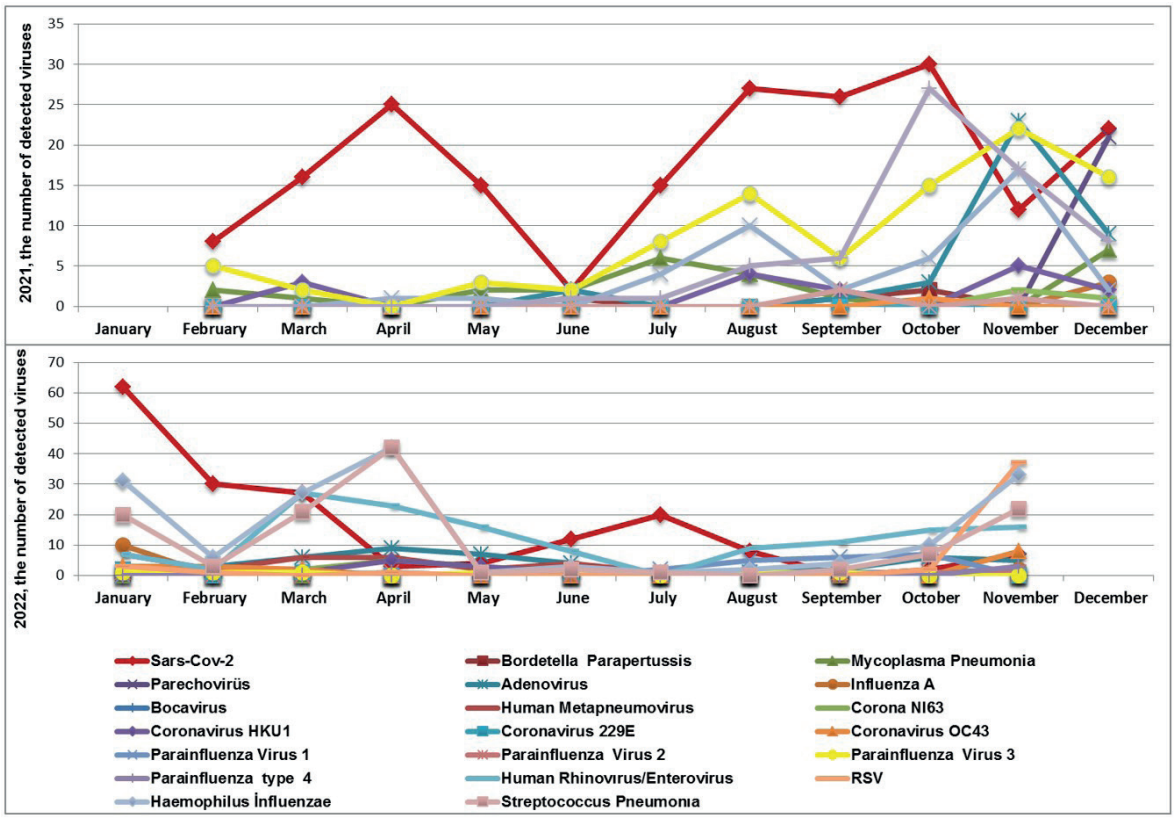


**Fig. 1.** Distribution of SARS-CoV-2 and other viruses by year and month.

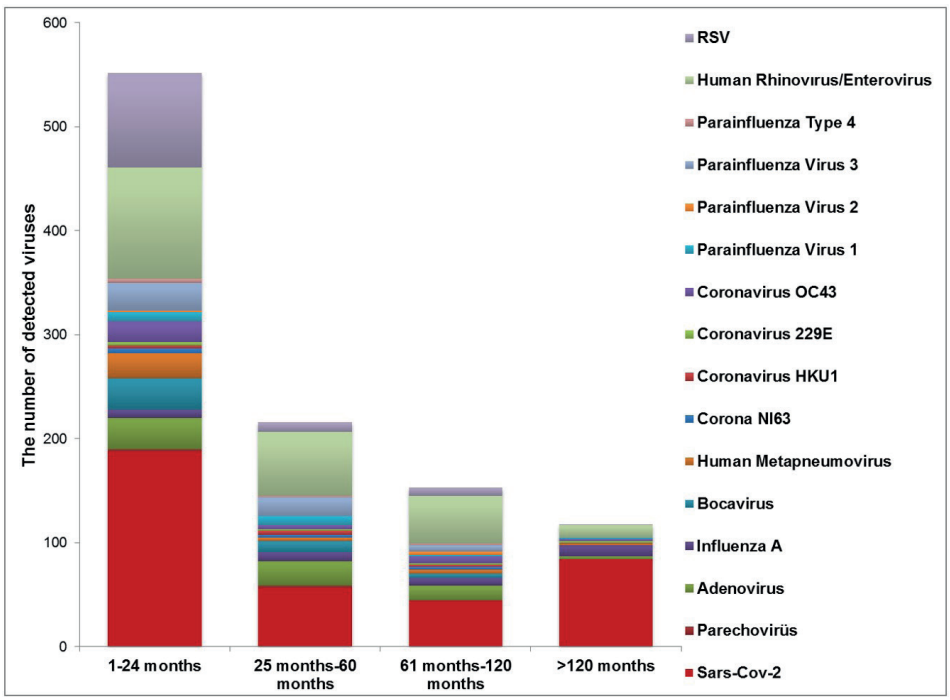


**Fig. 2.** Distribution of SARS-CoV-2 and other viruses by age.

In a total of 178 patients aged 121 months and older, 47.2% were positive for SARS-CoV-2, which was statistically significant. The distribution of SARS-CoV-2 and other viruses by age groups is shown in Fig. 2.

Co-infection with other respiratory viruses was detected in 46 of a total of 373 SARS-CoV-2 positive patients detected during the study period. In 38 patients, SARS-CoV-2 was co-infected with a single other respiratory virus, while in the remaining patients, two viruses were co-infected. The most common respiratory virus accompanying SARS-CoV-2 was HRV/EV in 18 patients, followed by RSV in 10 patients and influenza and human coronaviruses (OC43 in 5 and 229E in 1) in 6 patients each. Co-infection with other respiratory viruses was detected in 55 of 109 RSV-positive patients. In 43 patients, RSV was accompanied by a single virus, while at least two respiratory viruses were found in the remaining 12 patients. The most common concomitant virus in RSV-positive patients was HRV/EV, which was recorded in 22 patients. This was followed by HCoVs, which were positive in 14 patients (OC43 in 12 patients and 229E and NL63 in two patients). Interactions between respiratory viruses are shown in Table I.

During the study period, 386 patients were hospitalized for lower respiratory tract infections (acute bronchiolitis, pneumonia), 238 patients for upper respiratory tract infections (cryptic tonsillitis, sinusitis, acute otitis media, sinusitis, epiglottitis, croup), 202 patients for fever etiology, 111 patients for acute gastroenteritis and 236 patients for other diagnoses (such as convulsions, encephalitis, sepsis, rash, myocarditis, soft tissue infections, lymphadenopathy, eye infections).

During the study period, 113 patients (9.6%) required intensive care. The need for intensive care was statistically significantly higher for infections with Bocavirus and Respiratory Syncytial Virus between 1 month and 24 months, in the period July 1, 2021-July 1, 2022 (the first period when schools were held full-time face-to-face at all levels).

## Discussion

The development and worldwide dissemination of effective vaccines and the development of a certain level of immunity to the disease among people have led to a reduction in the frequency of infections caused by SARS-CoV-2 and the gradual withdrawal of bans.[6] In our country,

**Table I.** Interactions between respiratory viruses.

| Virus | Co-Infections | | | | Numbers | | |
|---|---|---|---|---|---|---|---|
| | None | Only one | Two or more | Most commonly detected virus | Total | 2021 | 2022 |
| SARS-CoV-2 | 327 (87.6%) | 38 | 8 | HRV/EV | 373 | 198 | 175 |
| HRV/EV | 133 (58.6%) | 74 | 20 | AoV | 227 | 93 | 134 |
| RSV | 54 (49.5%) | 43 | 12 | HRV/EV | 109 | 65 | 44 |
| HPIV (1,2,3,4) | 41 (50.6%) | 35 | 5 | HRV/EV | 81 | 47 | 34 |
| AoV | 30 (42.8%) | 29 | 11 | HRV/EV | 70 | 25 | 45 |
| HCoV | 18 (30.5%) | 27 | 14 | HRV/EV | 59 | 17 | 42 |
| HBoV | 18 (40%) | 20 | 7 | RSV | 45 | 38 | 7 |
| Influenza | 22 (62.8%) | 11 | 2 | SARS-CoV-2 | 35 | 21 | 14 |
| HMPV | 20 (62.5%) | 7 | 5 | HRV/EV | 32 | 3 | 29 |
| Parechovirus | - | 5 | - | RSV | 5 | 4 | 1 |

AoV: adenovirus, HBoV: human bocavirus, HCoV: human coronaviruses, HMPV: human metapneumovirus, HRV/EV: human rhinovirus/enterovirus, HPIV: human parainfluenza virus, RSV: respiratory syncytial virus, SARS-CoV-2: severe acute respiratory syndrome coronavirus-2.

the full removal of the bans took place on July 1, 2021. Starting with the 2021-2022 academic year, face-to-face education was provided to students at all levels.[7] In our study, the detection of viruses other than SARS-CoV-2 (66) was relatively low (28 respiratory viruses) before schools started face-to-face education. There was a significant increase in other respiratory viruses when schools started face-to-face education. During this period, while 270 SARS-CoV-2 viruses were detected, 487 were detected from other respiratory viruses. This is important because it shows that schools are a significant environmental factor for children in the spread of respiratory viruses.

Strict non-pharmaceutical measures taken worldwide have not only had an impact on SARS-CoV-2. They were also expected to have effects on other respiratory viruses transmitted directly or indirectly by contact or through airborne or droplet transmission. The debate on whether prolonged non-pharmaceutical measures affect community immunity to common pathogens and the circulation of viruses in the community has become more evident with recently published studies.[3,4]

In studies reported from Europe, the United States and Canada, a decrease of 45-70% was observed in emergency room admissions for children in the few months following the first COVID-19 case detection or the declaration of the pandemic.[8-14] In the two-month period after the pandemic was declared and the first case was seen in our country, on March 11, 2020, a significant decrease was detected in our hospital emergency room admissions compared to the same periods of previous years. For the two months between March 11 and May 11, 33 893 visits were made in 2018, 44 859 visits were made in 2019, and 8082 visits were made in 2020 to our hospital's emergency outpatient clinic. We emphasize that the contribution of bans to these reductions is crucial, but they are not effective alone. We know that in the pre-pandemic period, admissions were made to pediatric emergency departments even for very mild diseases.[15] Especially in terms of our hospital, the visits to the green zone among pediatric patients were very high in the pre-pandemic period. While there were 613 green zone visits per day in 2018 and 919 green zone visits in 2019, it decreased to 224 visits in 2020. With the pandemic, the intense coverage of uncertainties about the disease in the visual and written media and the reports of the serious course of the disease, especially in adults, may have caused fear in parents. Due to this fear, we think that patients with non-severe illnesses were treated at home instead of coming to the hospital.

With the pandemic, it has been reported that there has been a dramatic decline in diseases caused by respiratory viruses in many countries around the world.[16-18] A study by Vittucci et al.[16] in the first year of the pandemic (September 2020 - February 2021) showed a significant reduction (around 80%) in illnesses caused by respiratory viruses. It was observed that influenza (0 patients) and RSV (5 patients) infections were almost eliminated. In a study reported by Ippolito et al.[17] between November 1, 2020 and February 28, 2021, there was an 80% decrease in pediatric patients hospitalized for lower respiratory tract infections compared to previous years, with no hospitalizations due to influenza and RSV. In another study reported from Italy, it was shown that hospitalizations due to acute respiratory tract infection decreased by 82.2% between March 9, 2020, and February 28, 2021, with the lowest detection of other respiratory viruses including RSV (4 cases) and HRV/EV (1 case).[18] According to the weekly influenza surveillance reports of our country, a significant decrease in respiratory tract viruses evaluated within the scope of Sentinel SARI Surveillance among inpatients due to severe acute respiratory infection (SARI) between April 2020 and December 2021 (which coincides with the first autumn and winter seasons after the pandemic) was detected.[19] In the first season after this pandemic, no influenza positivity was reported in our country as in most countries, a decrease of over 99% in RSV-related infections was detected and 1 case was

reported.[19] In our hospital, a significant decrease in hospitalizations due to lower respiratory tract infectious diseases caused by respiratory viruses other than COVID-19 was observed during this period.

During the initial period of the pandemic, there were significant changes in the seasonal dynamics of respiratory viruses due to several factors, most notably the strictly implemented non-pharmaceutical measures. In many countries, a decline in the circulation of most respiratory viruses other than HRV/EV was observed.[16-20] The magnitude, timing, and duration of this reduction varied by respiratory virus. Historically, the lowest levels were detected in influenza virus and RSV infections.[16,19,21,22] These low levels did not persist in the second year of the pandemic as non-pharmaceutical measures were relaxed.[23-25] In a study conducted in France, it was observed that respiratory tract viruses were detected intensively. HRV/EV was detected most frequently, followed by RSV, AoV, HPIV types and HCoV types, respectively. Compared to previous periods, RSV had its first peak unexpectedly between February-April 2021 and its second significant wave between November 2021-January 2022. The influenza virus started to circulate in the 38$^{th}$-40$^{th}$ weeks and peaked in the 51$^{st}$ week.[23] In a study reported from Slovenia, almost all viruses were observed to return between April 2021 and March 2022. This is explained by the complete removal of bans, and the opening of schools. The RSV peak detected in September and October 2021 was earlier than in previous years. Influenza A infections were detected later than expected. HBoV1 was found to be high in April, May, June and July, HPIV types in May and June, HCOV types in February and March, AoVs were detected almost all year round, and HRV/EV was detected at the highest levels in September, March and July, but above certain levels throughout the year.[24] Another study reported from Germany examined the seasonal dynamics of respiratory viruses between January 2016 and January 2020. It was found that the detection rates of HBoV, HCoV, HPIV, and RSV viruses decreased during periods when non-pharmacological measures were intensively implemented. Unlike other seasonal viruses, INV, HMPV and HRV/EV remained at low levels even after the measures were removed. After the restrictions were lifted, an unseasonal increase in HBoV and RSV, a peak in HPIV detection rate delayed by two months compared to the pre-pandemic period, and a peak in HCoV detection with a delay of several months were observed.[25] In our study, no influenza virus was detected until December 2021. In 2021, RSV was observed for the first time in 1 patient in June and 1 patient in July, and increased from August onwards, the highest number of patients (27 patients) was reached in October and was statistically significant, then decreased and disappeared throughout the winter. In 2022, RSV was most common in November. HBoV was detected in a small number of patients in June, September, and October 2021, but the statistically greatest rates were found in November. The number of cases continued to decline in December and was detected sporadically throughout the study period in 2022. HMPV was detected in 3 patients only in December 2021, and in 2022, it continued to be seen until August, with the highest rates in January, March, and April. Among the HPIV types, especially type 3 was observed significantly in the summer and fall of 2021. In 2021, the highest rate was detected in November, with the second highest rate in August. While HPIV Type 1 was not seen at all in 2021, it was seen in the summer and fall of 2022. HPIV type 4 was detected in 3 patients each in the fall in both 2021 and 2022, while type 2 was detected in 1 patient only in October 2021 and in a total of 4 patients in all seasons except summer in 2022. Adenovirus was found throughout the year, more frequently in 2022. Among HCoV types, OC43 was observed in the summer and fall of 2021 and in winter 2022, while NL63 and HUK1 were not observed in 2021, but were observed in spring 2022. HRV/EV, on the other hand, was detected in all four seasons in 2021 and 2022, peaking in the fall and spring in both years.

In studies reported from many countries, HRV/EV were shown to be less affected by non-pharmaceutical measures taken against COVID-19.[17,18,20] In a study conducted in Türkiye within the first year of the pandemic, HRV/EV was the most frequently detected virus, even more frequently than SARS-CoV-2.[26] In our study, HRV/EV and AoV were less common in circulation with SARS-CoV-2 before the complete cessation of the restrictions. Shortly after, a significant increase in HRV/EV, HBoV, RSV, HCoV OC43, and HPIV was observed. A study reported from Germany found that HBoV, HCoV, HPIV, and RSV viruses showed an unseasonal increase in the period when measures were removed, similar to ours.[25]

The fact that viruses are non-enveloped makes them more resistant to external environments and allows them to live longer on surfaces. This allows them to be transmitted through direct or indirect contact. It has also been reported that non-enveloped viruses can pass through surgical masks.[27] For these reasons, especially HRV/EV has found a place in circulation in most countries even in the first period of the pandemic. However, in most reported studies, including our study, HBoV has not been shown to circulate like HRV/EV in the first year of the pandemic. We think that this may be related to viral interference. When the host is infected with more than one virus, complex interactions between these viruses are likely to occur. Among different virus-virus interactions, so-called viral interference is the direct or indirect antagonistic effect of one virus against another virus. It can do this by affecting the ability of the other virus to cause infection or disease in the host. Competition between viruses for resources can occur, leading to the down-regulation of receptors required for intracellular entry in the host, or by stimulating the innate immune response and interferon release.[4] In the 2009 H1N1 pandemic, such an association between influenza and HRV was observed.[28] A similar association was also shown in a study that found that previous HRV infection prevented SARS-CoV-2 replication by accelerating the responses of interferon-stimulated genes in the upper respiratory tract.[29] The influenza virus has remained low throughout the pandemic, even at its historically lowest level in most countries. Even when the measures were relaxed or even removed, and the expected peak did not occur. This was also observed in our study. The failure of influenza to reach its expected peak during the first year of the pandemic and even after the relaxation of bans may be due to viral interference with SARS-CoV2 or other respiratory viruses.

Following the lifting of restrictions around the world, it was observed that seasonal viruses did not follow the seasonal pattern they exhibited in the pre-pandemic period. At the end of 2020, an increase in inter-seasonal RSV infection rates was reported in Australia. In the same report, an increase was found in RSV infections between 2-4 years of age.[30] A study reported from Slovenia revealed that an early RSV epidemic peak was observed between September and October 2021. In addition, more hospitalizations occurred between the ages of 0-4 years compared to previous years.[24] RSV activity has increased since April 2021 in the publication reported from the United States. In some areas, there was an increase at an unexpected time.[21] While RSV was not seen in Tokyo in 2020, the largest increase in RSV cases occurred in 2021.[31] In our study, the average age of patients found to be RSV-positive was 14.4 months (1-146 months). The patient with the oldest age was SARS-CoV-2-positive and had a co-infection. Twenty-seven patients (24.7%) who were found to be RSV-positive received intensive care support. The mean age of patients receiving intensive care support was 8.7 months (1-86 months) and 70% of the patients were 4 months or younger. Co-infection with HBoV was detected in 4 of the RSV-positive patients requiring intensive care. We think that the increase in the rate of RSV infection at unexpected times and in an older age group and again the higher need for intensive care may be due to a lack of contact with RSV infection during pregnancy and early childhood, and immune-debt occurring in

children as a result of decreased immunity in older children and in the general population.

This study has some limitations. First of all, this was a single-center retrospective study. The hospital where the study was conducted is one of the many hospitals in the city where pediatric patients are hospitalized and followed up. The lack of data from other hospitals shows that the results will not cover the whole city. However, it can give a rough idea. The study does not include almost the first year of the pandemic, as respiratory tract multiplex PCR was not performed in our hospital. Therefore, data on other respiratory viruses are missing in almost the first year of the pandemic when public health measures were intensively implemented. In addition, evaluation of respiratory tract viruses by multiplex PCR was performed in our hospital approximately one year after the pandemic. Therefore, we could not make a comparison with the pre-pandemic period due to the lack of data from the years before the pandemic in our hospital. However, it is important that it includes the 5 months before the full removal of bans and the period immediately after. Since only inpatient data were used in our study, the results cannot be attributed to the entire population. However, due to the concerns of families early in the pandemic, more young children were hospitalized for follow-up due to COVID-19, leading to mild illnesses in hospitalization diagnoses.

Although it was concluded that with the removal of the bans, infections with other respiratory viruses, especially PIV, RSV and BoV, increased significantly earlier than the expected months when they were frequently detected before the pandemic, RSV and BoV-associated infections led to higher intensive care needs, even HRV/EV-associated infections led to intensive care needs, and influenza virus-associated infections did not increase as expected, it will be important to confirm this with more comprehensive prospective studies and to compare it with the pre-pandemic period with multicenter data.

**Ethical approval**

All procedures, including the informed consent process, were conducted per the National Health and Medical Research Council of Turkey's ethical standards and the Helsinki Declaration. The ethical committee of Prof. Dr Cemil Tascioglu City Hospital approved the study (No:2022/315). Informed consent was obtained from participants.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: AK, DKİ, ÖK; data collection: AK, ÇK, DKİ, ÖK, IE, LB, AB, GAT, EA; analysis and interpretation of results: AK, ZA; draft manuscript preparation: AK, DKİ, ÖK, AB. All authors reviewed the results and approved the final version of the manuscript.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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Original Article

# Association of SH2 domain-containing protein 1A, immunoglobulins and T lymphocyte subsets with Epstein-Barr virus infections

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## ABSTRACT

**Background.** We aimed to analyze the levels and associations of SH2 domain-containing protein 1A (SH2D1A), immunoglobulins and T lymphocyte (TL) subsets in children with Epstein-Barr virus (EBV) infections.

**Methods.** Sixty children with EBV infections admitted from January 2019 to January 2022 were selected, including 29 cases of infectious mononucleosis (IM group) and 31 cases of chronic active EBV infections (CAEBV group). Another 42 healthy children undergoing physical examination in the same period were selected as a control group. Their changes in SH2D1A, immunoglobulins and TL subsets (CD3+, CD4+ and CD8+) were compared.

**Results.** The levels of CD3+, CD4+ and CD8+ in the IM group were higher than those of the control group (P<0.05), while they were lower in the CAEBV group than those of the control and IM groups (P<0.05). The levels of SH2D1A, signaling lymphocyte activation molecule (SLAM) and SLAM-associated protein (SAP) were significantly higher in the IM group than those in the control and CAEBV groups (P<0.05). The CAEBV group had decreased protein expressions of SLAM and SAP compared with those of the IM group. SH2D1A was positively correlated with immunoglobulin A, immunoglobulin G and TL subsets (CD3+, CD4+ and CD8+) (P<0.05).

**Conclusions.** Detecting SH2D1A, immunoglobulins and TLs contributes to the diagnosis and differentiation of IM and CAEBV.

**Key words:** clinical diagnosis, Epstein-Barr virus, immunoglobulins, SH2 domain-containing protein 1A, signaling lymphocyte activation molecule, SLAM-associated protein, T lymphocyte subsets.

Epstein-Barr virus (EBV) was first found in the cell culture of malignant lymphoma in African children. As a double-stranded DNA virus of the human herpes virus (HHV) family, EBV is the first human B-lymphotropic virus found to be closely related to tumor occurrence and progression.[1] Humans are the only host of HHV and are generally susceptible, HHV is mainly transmitted through the oral-oral route.[2] As reported in previous studies, the positivity rate of serum anti-EBV antibodies in adults is over 95%, and most people are infected in childhood and become virus carriers for life.[3] In China, the positivity rate of anti-EBV antibody is up to 80.7% in children aged 3-5 years old, and >90% of children are seropositive by the age of 10.[4] After being infected with EBV, individuals can establish latent infections in human memory B lymphocytes. Stable immune functions can be obtained without the onset or clinical symptoms of disease in most adults, while the immune functions of children, especially infants, are still in the developmental stage. Therefore, children are more prone to deterioration and death than adults.[5]

Children with EBV infections may have no symptoms or mild respiratory symptoms. Infectious mononucleosis (IM) is the typical

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manifestation of initial EBV infections, which is an immunopathological and self-limiting disease. Most children with IM have a good prognosis and low mortality rates after symptomatic treatment, but systemic complications may occur in a few children with the progression of disease, thus causing severe IM.[6] EBV infections can also progress to lymphoma and chronic active EBV infections (CAEBV), often leading to a poor prognosis. The pathogenesis of CAEBV remains unclear, but the clonal expansion of EBV-infected T cells and NK cells has recently been implicated in the progression of CAEBV.[7,8]

Located at the X chromosome q25, SH2 domain-containing protein 1A (SH2D1A) is mainly present in activated T cells and NK cells, and its gene mutation/deletion can result in fatal EBV infections and EBV-associated hemophagocytic syndrome.[9-11] Moreover, signaling lymphocyte activation molecule (SLAM) plays an important role in T/B lymphocyte proliferation by enhancing T/B cell activation. SLAM-associated protein (SAP) encoded by SH2D1A can regulate different cells including T, NK and B lymphocytes in the immune system to exert normal immune functions.[12,13] SAP is required for NK cytotoxicity, T/B cell interaction, and T cell-dependent humoral immune responses. However, SAP deficiency affects SLAM-mediated levels of T/B lymphocytes, and leads to loss of function and excessive amplification of activation signals, ultimately causing the massive proliferation of B lymphocytes.[14,15]

Consequently, the levels of SH2D1A, immunoglobulins and T lymphocyte (TL) subsets in EBV-infected children were measured and compared with those in healthy children, aiming to clarify the pathogenesis of EBV infections and to provide references for clinical diagnosis and treatment.

## Material and Methods

### *General data*

This study has been approved by the ethics committee of our hospital, and written informed consent has been obtained from the caregivers of all children. Sixty children with EBV infections admitted from January 2019 to January 2022 were selected, including 29 cases of IM (IM group) and 31 cases of CAEBV (CAEBV group). The diagnostic criteria for IM were as follows: Fever, sore throat and hoarseness, hepatosplenomegaly, cervical lymphadenopathy >1 cm, peripheral blood atypical T cells >10, and EBV-VCA-immunoglobulin M (IgM) positivity. There were 16 boys and 13 girls aged 0.30-11.25 years old, with the mean of (4.25±1.24) years in the IM group. CAEBV was diagnosed based on the criteria of Hue et al.[16]: Persistent or recurrent IM-like symptoms such as fever, lymphadenopathy and hepatosplenomegaly, abnormal anti-EBV antibody levels (*i.e.* elevated anti-VCA/anti-early antigen [EA] antibody titers), and an increase in EBV-DNA copy number or EBV-DNA-positive tissues. There were 17 boys and 14 girls aged 0.45-11.09 years old, with a mean of (4.22±1.09) years in the CAEBV group. Additionally, healthy subjects undergoing physical examination during the same period were selected as the control group. After the cases with EBV infections and history of immunosuppressant use within 3 months were excluded, 42 children were finally enrolled, including 21 boys and 21 girls aged 0.37-11.15 years old, with a mean of (4.25±1.06) years. The gender and age compositions had no significant differences and were comparable among the three groups (P>0.05) (Table I).

### *Sample collection and treatment*

Before antiviral therapy and/or immune therapy in the hospital, fasting blood samples were drawn from children at 7 a.m., and placed into EDTA-anticoagulated tubes. One portion of the sample was treated to generate serum for ELISA, antibody dilution test and hemagglutination (HA) test. The other portion was used to isolate peripheral blood mononuclear cells (PBMCs). Then PBMCs were added to the total RNA extraction reagent TRIzol and stored in a refrigerator at -80°C.

**Table I.** Baseline clinical data.

| Group | n | Gender | | Age (year) | Clinical symptoms at admission | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Boy | Girl | | Fever | Sore throat | Cervical lymphadenopathy | Hepatosplenomegaly | Eyelid edema |
| Control | 42 | 21 | 21 | 4.25±1.06 | | | | | |
| IM | 29 | 16 | 13 | 4.25±1.24 | 23 | 28 | 29 | 15 | 23 |
| CAEBV | 31 | 17 | 14 | 4.22±1.09 | 28 | 26 | 30 | 17 | 27 |
| P | | 0.883 | | 0.241 | 0.233 | 0.102 | 0.329 | 0.809 | 0.419 |

CAEBV: Chronic active Epstein-Barr virus, IM: infectious mononucleosis.

### *TL subset assay*

TL subsets including $CD3^+$, $CD4^+$ and $CD8^+$ were detected using the FC50 flow cytometer (BC, USA) according to the manufacturer's instructions. The supporting software CXP2.0 system was used for image analysis.

### *Immunoglobulin assay*

The levels of IgA, IgG and IgM in the serum obtained by venous blood centrifugation were detected by immuno-scatter turbidimetry using the kits purchased from Siemens (Germany).

### *Quantitative RT-PCR*

The primer amplification conditions were optimized in the pre-experiment. PCR amplification (a total reaction volume of 20 μL) was repeated 3 times in strict accordance with the instructions of the SsoFast EvaGreen RT-PCR kit. The relative expression of SH2D1A mRNA was calculated by ΔΔC1.

### *Western blotting*

NK cells ($CD3^-CD16^+CD56^+$) in the 6-well plate were washed once with PBS, digested with 500 μL of 0.25% trypsin-EDTA, and placed in EP tubes. After digestion was terminated by 1.5 mL of complete medium, the cells were thoroughly pipetted, and 250 μL of protein lysis buffer were added, followed by an ice bath for 30 min, during which the tube was shaken every 10 min. Following centrifugation at 12,000 r/min and 4°C for 10 min, the supernatant (total cell protein) was harvested and stored in a refrigerator at -40°C for later use. In strict accordance with the BCA protein assay kit, 10% separation gel and 5% spacer gel were prepared, and the protein sample was loaded, subjected to electrophoresis at 110 V for 100 min, and transferred onto an NC membrane. The membrane was then blocked, followed by incubation with primary antibodies at 4°C overnight, washing, incubation at room temperature for 2 h, and washing again. Finally, the image was developed with a gel imager.

### *Statistical analysis*

SPSS 25.0 software was used for statistical analysis. The measurement data were expressed as ($\bar{x} \pm s$), and compared by the *t*-test. The count data were expressed as rate (%), and compared by the $\chi^2$ test. Spearman's rank correlation analysis was conducted. P<0.05 was considered statistically significant.

## Results

### *Immunoglobulin levels*

There were significant differences in IgA and IgG among the three groups (P<0.05). The levels of IgA and IgG in the IM and CAEBV groups were significantly higher than those in the control group (P<0.05). The IgM level had no significant difference between the IM and CAEBV groups (P>0.05) (Table II).

### *Peripheral blood TL subsets*

There were significant differences in TL subsets ($CD3^+$, $CD4^+$, and $CD8^+$) among the three groups (P<0.05). The levels of $CD3^+$, $CD4^+$ and $CD8^+$ in the IM group were higher than those in the

**Table II.** Immunoglobulin levels.

| Group | n | IgA (g/L) | IgG (g/L) | IgM (g/L) |
|---|---|---|---|---|
| Control | 42 | 2.59±0.67 | 9.69±2.15 | 1.31±0.41 |
| IM | 29 | 5.42±1.65[a] | 14.27±3.98a | 1.39±0.42 |
| CAEBV | 31 | 5.13±1.95[a] | 14.17±4.47a | 1.29±0.39 |
| P | | <0.001 | <0.001 | 0.825 |

CAEBV: Chronic active Epstein-Barr virus, Ig: immunoglobulin, IM: infectious mononucleosis. [a]P<0.05 vs. control group.

**Table III.** Expressions of SH2D1A mRNA and related proteins.

| Group | n | SH2D1A mRNA | SLAM | SAP |
|---|---|---|---|---|
| Control | 42 | 5.65±2.67 | 1.88±0.26 | 0.26±0.12 |
| IM | 29 | 38.42±4.65[a] | 4.38±0.31[a] | 0.61±0.14[a] |
| CAEBV | 31 | 5.13±3.95[a] | 1.91±0.30[a] | 0.25±0.13a |
| P | | <0.001 | <0.001 | <0.001 |

CAEBV: Chronic active Epstein-Barr virus, IM: infectious mononucleosis, SAP: SLAM-associated protein, SH2D1A: SH2 domain-containing protein 1A, SLAM: signaling lymphocyte activation molecule. [a]P<0.05 vs. control group.

control group (P<0.05), while they were lower in the CAEBV group than those in the control and IM groups (P<0.05) (Fig. 1).

### *Expressions of SH2D1A mRNA and related proteins*

Statistically significant differences were found in the levels of SH2D1A, SLAM and SAP among the three groups (P<0.05). The levels of SH2D1A, SLAM and SAP were significantly higher in the IM group than those in the control and CAEBV groups (P<0.05), while they had no significant differences between the control and CAEBV groups (P>0.05) (Table III).

### *Western blotting results of SH2D1A-related proteins*

SH2D1A-related protein SLAM- and SAP-specific pre-stained protein bands were found at about 36, 130 and 20 kDa. The results of Western blotting showed that the expressions of SLAM and SAP were consistent with each other. Compared to the control group, the protein bands were obviously wider and most strongly stained, and the protein expression was up-regulated in the IM group. The CAEBV group had lightly stained protein bands and decreased protein expressions of SLAM and SAP compared with those of the IM group (Fig. 2).

### *Results of correlation analysis of immunoglobulins, TL subsets and SH2D1A mRNA expression*

The change trends of immunoglobulins, TL subsets and SH2D1A mRNA expression were similar. According to Spearman's analysis, SH2D1A expression was positively correlated with IgA, IgG and TL subsets ($CD3^+$, $CD4^+$ and $CD8^+$) (P<0.05), whereas no significant correlation was found between IgM and SH2D1A mRNA expression (P=0.0833) (Fig. 3).

## Discussion

The occurrence and progression of EBV infections depend primarily on the TL-mediated cellular immune response of the human immune system. $CD3^+$, $CD4^+$ and CD8 are the surface markers of T cells, effector T cells, and cytotoxic and suppressor T cells, respectively. Therefore, EBV infections are closely related to $CD3^+$, $CD4^+$ and $CD8^+$. The immune system is normally in a balanced state in two ways. On the one hand, suppressor T cells inhibit the activation of effector B cells and T cells, weakening the immune function of the human body. On the other hand, Th cells enhance the activation of B cells and T cells by releasing cytokines, thereby strengthening the immune function. A nationwide survey in

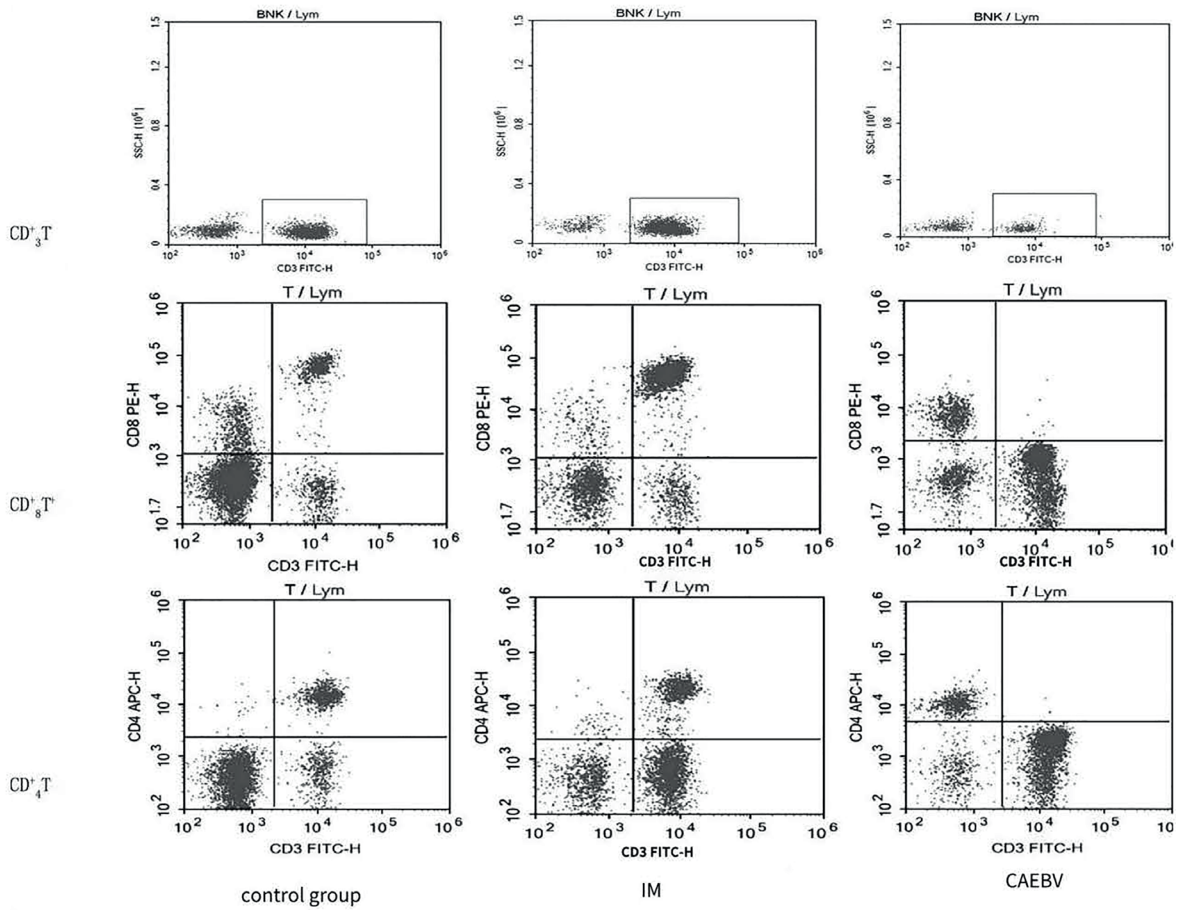


**Fig. 1.** Flow cytometry results of peripheral blood TL subsets.

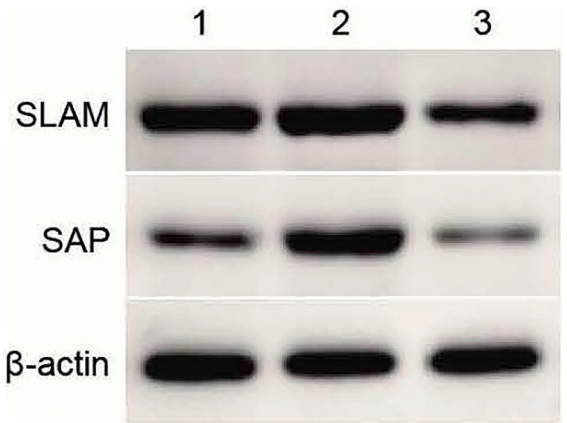


**Fig. 2.** Western blotting results of SH2D1A-related proteins SLAM and SAP. 1: Control group. 2: IM group. 3: CAEBV group.

Japan showed that CAEBV occurred in most of the 82 patients and its pathogenesis involved either T cells or NK cells, while B cells were involved in infections in only 2 patients.[17] After EBV infections occur, the intestinal mucosa and digestive system secrete a large number of immunoglobulins, so the body is in an infection-specific sensitization state and the immunoglobulin levels significantly increase. As a result, a series of biochemical reactions occur to prevent viral infection in the case of exposure to EBV antigens again. However, the association between humoral immunity and severity of EBV infections is unclear yet.[18,19]

In this study, the levels of $CD3^+$, $CD4^+$ and $CD8^+$ in the peripheral blood were significantly higher in IM children. Hence, strong TL reactions occur following EBV infections in IM children, thus leading to massive proliferation of TLs and abnormal levels of peripheral blood TLs. $CD8^+$ TL proliferation and activation can be found in about 80% of typical IM cases. In this

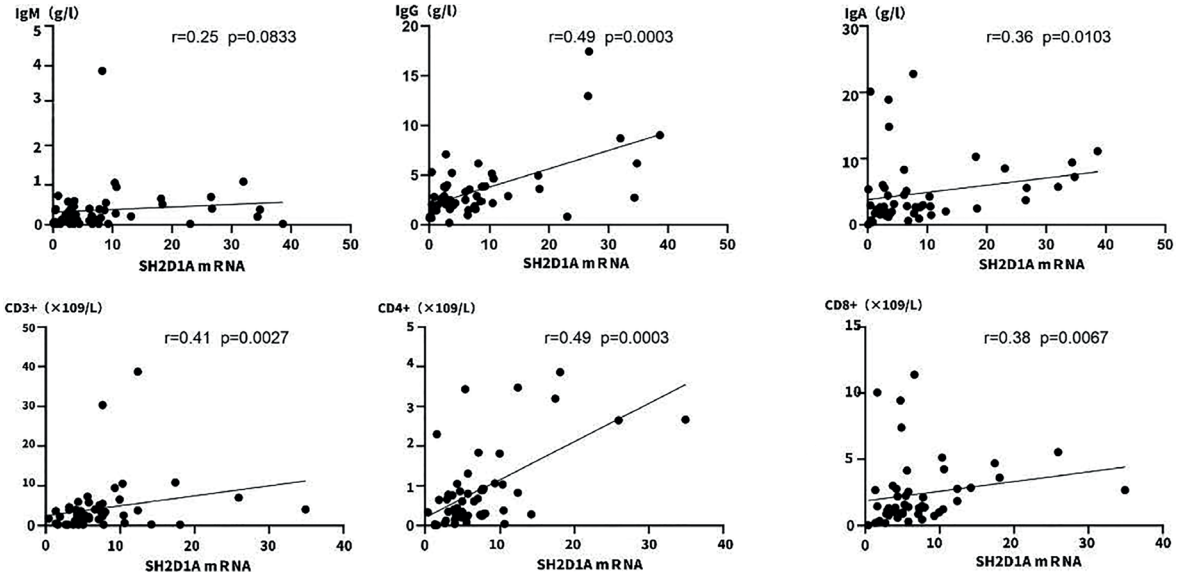


**Fig. 3.** Results of correlation analysis of immunoglobulins, TL subsets and SH2D1A mRNA expression.

study, the CAEBV group had lower levels of $CD3^+$, $CD4^+$ and $CD8^+$ than those of the control and IM groups ($P<0.05$). Probably, CAEBV children cannot control or eliminate EBV, and suffer from immune system disorders due to EBV immunodeficiency, which is the main mechanism of EBV infections progressing to CAEBV.

Upon stimulation by EBV, specific antibodies such as IgA, IgG and IgM antibodies are produced in lymphocytes and then involved in the immune response, resulting in associated immune expression.[20] In this study, the levels of IgA and IgG in IM and CAEBV groups were significantly higher than those in the control group ($P<0.05$), indicating that after infection with EBV, the cellular and humoral immunity became disordered, the levels of IgG and IgM increased, and immune imbalance occurred. The findings are consistent with those of Ye et al.[21]

We also found that the expressions of SH2D1A mRNA and related proteins SLAM and SAP significantly rose in IM children after EBV infections occurred, indicating severe cellular immune dysfunction. In contrast, the expressions of SH2D1A mRNA, SLAM and SAP had no obvious changes and were even at low levels in the CAEBV group. Unknown host genetic predisposition may be associated with the onset of CAEBV, and different characteristics of peripheral blood SH2D1A variants in CAEBV and IM children are of significance for early identification and diagnosis. Therefore, close attention should be paid to the possibility of delayed onset, persistent EBV infections and tumor progression in patients with significant decreases in the expressions of SH2D1A mRNA, SLAM and SAP. In addition, SH2D1A mRNA expression was positively correlated with IgA, IgG, $CD3^+$, $CD4^+$ and $CD8^+$ in children with EBV infections, suggesting that the expressions of SH2D1A and related proteins SLAM and SAP can reflect the immune function of EBV infections, as new immune-related molecular markers for the clinical diagnosis of IM and CAEBV.

In conclusion, the SH2D1A mRNA level in the peripheral blood significantly rises in children with EBV infections, which cause disorders of humoral immunity and cellular immunity. $CD8^+$ lymphocytes markedly proliferate in IM children, but lymphocytes decrease in CAEBV children. IM and CAEBV children have significantly increased levels of IgA and IgG.

The detection of SH2D1A, immunoglobulins and TLs contributes to the clinical diagnosis and differentiation of IM and CAEBV.

## Ethical approval

This study has been approved by the ethic committee of our hospital, and written informed consent has been obtained from the caregivers of all children. The study has received ethical approval by Children's Hospital of Nanjing Medical University (202004024-1).

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: PX, JZ, HM; data collection: PX, JZ, YX; analysis and interpretation of results: PX, JZ, YX; draft manuscript preparation: HM. All authors reviewed the results and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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Original Article

# Pattern of hereditary renal tubular disorders in Egyptian children

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## ABSTRACT

**Background.** Hereditary renal tubular disorders (HRTD) represent a group of genetic diseases characterized by disturbances in fluid, electrolyte, and acid-base homeostasis. There is a paucity of studies on pediatric HRTD in Egypt. In this study, we aimed to study the pattern, characteristics, and growth outcome of HRTD at an Egyptian medical center.

**Methods.** This study included children from one month to < 18-years of age with HRTD who were diagnosed and followed up at the Pediatric Nephrology Unit of Sohag University Hospital from January 2015 to December 2021. Data on patients' demographics, clinical features, growth profiles, and laboratory characteristics were collected.

**Results.** Fifty-eight children (57% males; 72% parental consanguinity; 60% positive family history) were diagnosed with seven HRTD types. The most commonly encountered disorders were distal renal tubular acidosis (distal renal tubular acidosis [RTA] 27 cases, 46.6%) and Bartter syndrome (16 cases 27.6%). Other identified disorders were Fanconi syndrome (6 cases with cystinosis), isolated proximal RTA (4 cases), nephrogenic diabetes insipidus (3 cases), and one case for each RTA type IV and Gitelman syndrome. The median age at diagnosis was 17 months with a variable diagnostic delay. The most common presenting features were failure to thrive (91.4%), developmental delay (79.3%), and dehydration episodes (72.4%). Most children showed marked improvement in growth parameters in response to appropriate management, except for cases with Fanconi syndrome. Last, only one case (with cystinosis) developed end-stage kidney disease.

**Conclusions.** HRTD (most commonly distal RTA and Bartter syndrome) could be relatively common among Egyptian children, and the diagnosis seems challenging and often delayed.

**Key words:** renal tubular disorders, renal tubular acidosis, Bartter syndrome, failure to thrive, dehydration.

Hereditary renal tubular disorders (HRTD) represent a heterogeneous group of rare genetic diseases of renal tubules, characterized by disturbances in fluid, electrolyte, and acid-base homeostasis. Patients with HRTD are exposed to life-threatening complications, such as dehydration episodes, seizures, hypokalemia, and metabolic acidosis. Moreover, HRTD are associated with long-term sequelae, including growth impairment, bone deformities, and chronic kidney disease (CKD).[1-3] In recent years, there has been an increasing interest in HRTD thanks to advances in diagnosis (e.g., molecular studies) and the advent of new therapeutic modalities, which remarkably improve patient outcomes.[4-6] The most commonly reported HRTD include distal renal tubular acidosis (dRTA), proximal renal tubular acidosis (pRTA), Bartter syndrome, idiopathic hypercalciuria, and X-linked hypophosphatemic rickets (XLHR). However, there are limited data on the exact prevalence of individual HRTD, and their relative frequencies vary among different studies and geographic regions. This calls for region-specific studies on the pattern and characteristics of HRTD.[4,6]

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Egypt is one of the largest countries in the Middle East and Africa, spanning over one million $Km^2$ and has >100 million population. There is a paucity of studies on HRTD among children in Egypt and developing countries. Given the high rate of consanguineous marriage (up to 60%), the prevalence of HRTD may be relatively higher among Egyptian children.[7] However, the diagnosis may be challenging due to the broad, nonspecific, and overlapping manifestations as well as poor pediatrician awareness and limited diagnostic capabilities.[1,8,9] In this study, we aimed to study the pattern, relative frequency, characteristics, and outcome of hereditary renal tubular disorder at an Egyptian medical center.

## Material and Methods

This study included children from one month to <18-years of age with hereditary renal tubular disorders who were diagnosed and followed up at the Pediatric Nephrology Unit of Sohag University Hospital (PNU-SUH) from January 2015 to December 2021. We excluded children with renal tubular disorders due to acquired causes, such as drugs (e.g., diuretics), intoxications (e.g., vitamin D), autoimmune disorders, and obstructive uropathy, as well as those with a follow-up duration of fewer than six months. The PNU-SUH has been established since 2005 as the main referral center for children with renal disorders in Sohag governorate, which is located in southern Egypt, spanning over 1,547 $km^2$, and has >5 million population. The present study was approved by the Medical Research Ethics Committee of Sohag Faculty of Medicine on April 11$^{th}$, 2021 (IRB Registration Number: Soh-Med-21-04-04) and followed the ethical guidelines of the 1964 Declaration of Helsinki and its 2013 revision. Informed consent was obtained from parents or authorized legal guardians of children participating in this study.

Enrolled children underwent thorough history taking and physical examination, including patients' demographics, perinatal history (e.g., polyhydramnios, prematurity, and admission to neonatal intensive care unit [NICU]), family history, age at presentation and diagnosis, and detailed manifestations (e.g., failure to thrive [FTT], delayed development, polyuria, polydipsia, episodes of dehydration, vomiting, seizures, rickets/bone deformities, photophobia, and deafness). Anthropometric measures and development were evaluated using WHO growth charts (percentiles and z-scores) and the Denver II developmental screening test (http://denverii.com), respectively. Laboratory investigations included complete blood count, hepatic and renal function tests, blood gases and anion gap estimation, serum levels of electrolytes, glucose, urine analysis and urinary chloride, sodium, anion gap, and calcium/creatinine ratio, estimation of glomerular filtration rate, renal Ultrasound, bone radiograph, bicarbonate loading test, and ammonium loading test as well as slit lamp examination for corneal cystine crystals. Following diagnosis, children received appropriate management according to guidelines with regular follow-up; data were obtained during serial follow-up visits.

Diagnosis of HRTA relied on characteristic clinical and biochemical data after exclusion of acquired causes. dRTA was diagnosed based on normal anion gap (hyperchloremic) metabolic acidosis, hypokalemia, inability to lower urinary pH below 5.5 during metabolic acidemia, and hypercalciuria. Isolated pRTA was diagnosed on the basis of hyperchloremic metabolic acidosis, no or mild hypokalemia, and preserved ability to lower urinary pH below 5.5 in the case of metabolic acidemia or acid loading. Fanconi syndrome was defined as pRTA with glucosuria, phosphaturia, and aminoaciduria. Cystinosis was defined as Fanconi syndrome with cystine crystals in the cornea and/or high cystine levels in leukocytes.

RTA type IV was diagnosed on the basis of hyperchloremic metabolic acidosis, hyperkalemia, and the ability to lower urinary pH below 5.5 in response to metabolic acidemia. Diagnosis of Bartter syndrome relied on a history of polyhydramnios/prematurity and hypochloremic hypokalemic metabolic

alkalosis with excessive urinary loss of calcium and chloride. Gitelman syndrome was defined as hyperchloremic metabolic alkalosis with hypocalciuria and hypomagnesemia. Nephrogenic diabetes insipidus (NDI) was diagnosed on the basis of polyuria, polydipsia, high plasma osmolarity concomitant with low urine osmolarity, persistent hypernatremia, high serum vasopressin, and no response to vasopressin test.[1,3,10-15]

The estimated glomerular filtration rate (eGFR) was calculated using the revised Schwartz formula for pediatric patients.[16] Chronic kidney disease (CKD) was defined according to Kidney Disease Improving Global Outcomes (KDIGO) criteria as permanent (> 3 months) eGFR < 60 ml/min/1.73 $m^2$.[17]

### *Statistical analysis*

Patient data were analyzed using IBM SPSS for Windows version 25 (IBM Corp., Armonk, NY, USA). Qualitative data were presented as frequency and percentages, and quantitative data were presented as medians and ranges (as the Shapiro-Wilk test showed that quantitative data were not normally distributed). We used Fisher exact test to compare frequencies of FTT and developmental delay between patients diagnosed during and after the first year of life. Height and weight measures were compared during follow-up using Wilcoxon test. A *p*-value (2-tailed) <0.05 was considered statistically significant.

## Results

Over seven years (2015 – 2021), 58 children (33 males and 25 females) from 54 unrelated families were diagnosed with HRTD at our medical center. One family had three siblings with Fanconi syndrome and two families each had two siblings with dRTA. Positive parental consanguinity and family history of similar conditions were present in 72.4% and 60.3% of cases, respectively. Nearly a third of cases (34.5%) were born prematurely (<37 weeks of gestational age), and 41.4% required NICU admission. The median age at diagnosis was 17 months (range 3 months to 7 years); around 45% of cases were diagnosed during the first year of life, while only two cases were diagnosed after the age of 6 years. The most commonly encountered disorders were dRTA (27 cases, 46.6%) and Bartter syndrome (16 cases, 27.6%). Other identified disorders were Fanconi syndrome (6 cases; all have cystinosis), isolated pRTA (4 cases), NDI (3 cases), and one case for each of RTA IV and Gitelman syndrome (Table I).

As shown in Table II, the most common presenting feature in children under study was FTT (91.4%), developmental delay (79.3%),

**Table I.** Pattern of hereditary renal tubular disorders among children.

| Category | Patients' n (%) | Male/Female | Consang., n (%) | Family history, n (%) | Age at diagnosis (months), median (IQR) | Follow-up duration (months), median (IQR) |
|---|---|---|---|---|---|---|
| dRTA | 27 (46.6) | 15/12 | 19 (70.4) | 14 (51.9) | 12 (6-24) | 12 (12-12) |
| Bartter syndrome | 16 (27.6) | 10/6 | 10 (62.5) | 10 (62.5) | 12 (7-31) | 12 (10.5-12) |
| Fanconi syndrome | 6 (10.3) | 3/3 | 6 (100) | 5 (83.3) | 17.5 (12-48) | 12 (12-12) |
| pRTA | 4 (6.9) | 2/2 | 3 (75) | 2 (50) | 36 (31-39) | 12 (12-15) |
| NDI | 3 (5.2) | 2/1 | 2 (66.7) | 2 (66.7) | 60 (29-84) | 12 (7-12) |
| Gitelman syndrome | 1 (1.7) | 1/0 | 1 (100) | 1 (100) | 60 | 36 |
| RTA IV | 1 (1.7) | 0/1 | 1 (100) | 1 (100) | 5 | 7 |
| Total | 58 | 33/25 | 42 (72.4) | 35 (60.3) | 17 (7-36) | 12 (12-12) |

dRTA: distal renal tubular acidosis, NDI: nephrogenic diabetes insipidus, pRTA: proximal renal tubular acidosis, RTA IV: renal tubular acidosis type IV

**Table II.** Main presenting features in children with renal tubular disorders.

| Category | Failure to thrive | Developmental delay | Dehydration episodes | Polyuria | Vomiting | Rickets | Skeletal deformity/ fracture | Nephrocalcinosis |
|---|---|---|---|---|---|---|---|---|
| dRTA (n=27) | 25 (92.6) | 23 (85.2) | 21 (77.8) | 19 (70.4) | 18 (66.7) | 17 (63) | 2 (7.4) | 13 (48.2) |
| Bartter syndrome (n=16) | 14 (87.5) | 12 (75) | 10 (62.5) | 10 (62.5) | 9 (56.3) | 10 (62.5) | 0 | 7 (43.8) |
| Fanconi syndrome (n=6) | 6 (100) | 5 (83.3) | 5 (83.3) | 4 (66.7) | 5 (83.3) | 4 (66.7) | 4 (66.7) | 0 |
| pRTA (n=4) | 4 (100) | 4 (100) | 3 (75) | 3 (75) | 4 (100) | 4 (100) | 3 (75) | 0 |
| NDI (n=3) | 2 (66.7) | 0 | 2 (66.7) | 3 (100) | 1 (33.3) | 0 | 0 | 0 |
| Gitelman syndrome (n=1) | 1 (100) | 1 (100) | 0 | 1 (100) | 0 | 1 (100) | 1 (100) | 0 |
| RTA IV (n= 1) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 | 0 |
| Total (n=58) | 53 (91.4%) | 46 (79.3%) | 42 (72.4%) | 41 (70.7%) | 38 (65.5%) | 37 (63.8%) | 10 (17.2) | 20 (34.5%) |

Data are described as n (%)
dRTA: distal renal tubular acidosis, NDI: nephrogenic diabetes insipidus, pRTA: proximal renal tubular acidosis, RTA IV: renal tubular acidosis type IV





**Table III.** Height and weight outcome assessment.

| Category | Age at diagnosis (months), median (IQR) | Age at last follow-up (months), median (IQR) | Height Z-score at diagnosis, median (IQR) | Height Z-score at follow-up, median (IQR) | p-value* | Weight Z-score at diagnosis, median (IQR) | Weight Z-score at follow-up, median (IQR) | P-value* |
|---|---|---|---|---|---|---|---|---|
| dRTA (n = 27) | 12 (6-24) | 24 (17-36) | -3.3 (-5.5, 0.2) | -2 (-3.8 : -1) | 0.005 | -4 (-5.4, -2.9) | -1.3 (-2.1, 0.3) | 0.000 |
| Bartter syndrome (n = 16) | 12 (7-31) | 23 (18-48) | -2.3 (-3.1, -1.6) | -2.15 (-3.2, -1.4) | 0.053 | -3.95 (-5.1, -3) | -0.7 (-2.1, 0.2) | 0.001 |
| Fanconi syndrome (n = 6) | 17.5 (12-48) | 30 (24-60) | -4.6 (-5.3, -4.1) | -4.8 (-5.3, -4.5) | 0.599 | -4.9 (-5.7, -4.2) | -4.9 (-5.2, -3.8) | 0.674 |
| pRTA (n = 4) | 36 (31-39) | 48 (43-54) | -3.9 | -4.15 | NA | -3.9 | -3.45 | NA |
| NDI (n = 3) | 60 (29-84) | 72 (36-96) | -4 | -3.2 | NA | -1.9 | 1.4 | NA |
| Gitelman syndrome (n = 1) | 60 | 96 | -2.4 | -2.3 | NA | -4.8 | -3.5 | NA |
| RTA IV (n = 1) | 5 | 12 | -3.9 | -4.15 | NA | -6.4 | -3.3 | NA |

dRTA: distal renal tubular acidosis, NA: not applicable, NDI: nephrogenic diabetes insipidus, pRTA: proximal renal tubular acidosis, RTA IV: renal tubular acidosis type IV
* Wilcoxon test

and dehydration episodes (72.4%). Compared with patients diagnosed in the first year of life, those diagnosed after the first year of life had higher proportions of FTT (100% vs. 80.8%, $p$ =0.014) and developmental delay (87.5% vs. 69.2%, $p$ =0.111). Other features included polyuria (70.7%), vomiting (65.5%), clinical and/or radiological signs of rickets (63.8%), nephrocalcinosis (34.5%), seizures (24.1%), and bone deformities with or without pathological fractures (17.2%). Hearing impairment and photophobia was identified in only one case with dRTA and cystinosis, respectively.

The assessment of height and weight outcomes is provided in Table III and Fig. 1A & 1B. Patients with dRTA showed statistically significant improvements in both height and weight. Patients with Bartter syndrome had improvements in both height and weight, but only weight improvement reached a statistically significant level. Other disorders showed no statistically significant difference in height and weight during the follow-up.

Our PNU-SUH follow recommended dietary and pharmacological guidelines in management of fluids, electrolytes, acid-base, and other disorders in children with HRTD.[3,14,15] However, access to medical services and medication as well as in-adherence to treatment were major challenges. Acid-base and electrolyte homeostasis could not be normalized in some cases, particularly those with pRTA and Fanconi syndrome. During the study duration, children under study had no significant changes in GFR, and only one case with cystinosis developed CRF and started renal replacement therapy.

## Discussion

Renal tubules play a crucial role in fluids, electrolytes, and acid-base homeostasis. Tubular dysfunction can result from a variety of hereditary and acquired causes and has to be considered in the differential diagnosis of children with failure to thrive, polyuria, refractory rickets, hypokalemia, and metabolic acidosis.[3] The present study investigated the pattern, characteristics, and outcome of 58 children with HRTD at an Egyptian medical center. These data advance our knowledge on HRTD in southern Egypt and are quite important for increasing pediatricians' awareness for early diagnosis and treatment, which is essential for a better outcome.

In the present study, the most commonly identified HRTD were dRTA and Bartter syndrome, which is consistent with some previous studies. For instance, dRTA and Bartter syndrome were the most frequent disorders in two Indian studies on children with HRTD.[18,19] A Turkish study on 226 patients with HRTD reported that the most common types

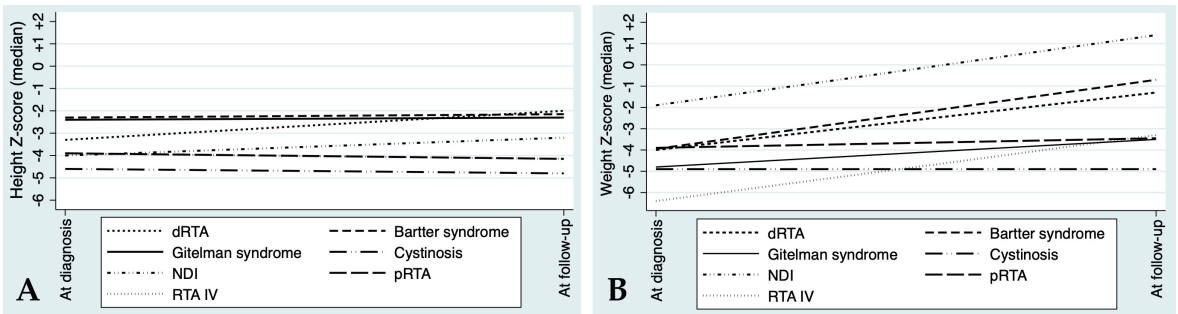


**Fig. 1. A.** Height z-scores at diagnosis and follow-up. **B.** Weight z-scores at diagnosis and follow-up

dRTA: distal renal tubular acidosis, NDI: nephrogenic diabetes insipidus, pRTA: proximal renal tubular acidosis, RTA IV: renal tubular acidosis type IV

were dRTA (45.6%), pRTA (26.6%), and Bartter syndrome (21.7%).[1] Based on the data of more than 200 patients mostly from Latin America and Spain, the Renal Tube Network reported that the most frequent tubulopathies are dRTA and Bartter syndrome.[20]

On the other hand, a German study reported that nephropathic cystinosis, XLHR, and idiopathic hypercalciuria are the most frequent HRTD.[21] In a Spanish study, Gitelman syndrome was the most common HRTD (36%), followed by dRTA (15%) and cystinosis (17%).[4] Idiopathic hypercalciuria (35%), followed by cystinosis and RTA (each 21.6%) were the most frequent HRTD among 37 children from Iraq.[22] Another Iraqi study on 80 patients reported that pRTA (56.3%), dRTA (30%), and Bartter syndrome (10%) are the most frequent HRTD.[23] In an Iranian study, the most commonly reported tubulopathies were RTA (33%), calcium disorders (27%), and cystic diseases (17%).[2] Our study didn't include certain types of HRTD, such as idiopathic hypercalciuria, which may be related to a referral bias, since most of these children may not be referred to a pediatric nephrology center.

The variability in the pattern and relative frequency of HRTD among studies can be attributed to a variety of reasons. First, true differences in birth prevalence of HRTD may occur among certain populations. Second, studies have different inclusion and exclusion criteria. For example, Hooman et al.[2] included both hereditary and acquired tubulopathies, while Topaloglu et al.[1] and Azat[23] studies excluded patients with NDI, idiopathic hypercalciuria, cystinuria, and stone diseases such as hyperoxaluria. Third, there are differences in the level of physician awareness and available diagnostic facilities, which may preclude diagnosis of certain HRTD in resource-limited areas. Last, most studies have a small sample size, which may not properly reflect the real pattern of HRTD.

Children in our study were diagnosed at a median age of 17 months with a range from three months to seven years. This is close to Kiran et al.[18] study, in which the median age at diagnosis was 18 months. Some studies reported an earlier median age at diagnosis, such as 12 months in the Azat[23] study, while other studies reported later diagnosis, such as four years in Sinha et al.[19] study and five years in Blázquez Gómez et al.[4] study. It is important to note that patients with more severe disorders, such as dRTA, Bartter, and Fanconi syndrome, were diagnosed at an earlier age than that of patients with disorders of more silent course, such as Gitelman syndrome. Moreover, there was a notable delay between the onset of symptoms and diagnosis, which has been also reported in some previous studies, particularly in developing countries.[1,8] The diagnostic delay could be attributed to a delay in seeking medical advice, heterogenous clinical manifestations, improper physicians' awareness, and limited diagnostic facilities. Indeed, a survey study of attendees to the Spanish Nephrology Society Congress in 2019 revealed inadequate knowledge on dRTA.[9]

The general percentage of consanguineous marriage in Egypt is 35%, but it reaches 60% in rural areas.[7] In the present study, positive parental consanguinity and family history of similar conditions were present in 72.4% and 60.3%, respectively, of children with HRTD. A high proportion of consanguinity has been reported in previous studies from the Middle East, such as 77% in Topaloglu et al.[1], and 85% in Azat[23] and a high proportion of other affected family members have been reported, such as 58.8% in Azat's study.[23] The high proportions of consanguinity and other affected family members indicate a high incidence of HRTD in Egypt. When this is combined with the observed diagnostic delay, it is highly likely that many other patients with HRTD remain undiagnosed. This also underscores the importance of screening other family members after confirming the diagnosis of the index patient, which may identify other patients so that management can be started early to prevent possible acute and long-term complications.

The most common presenting manifestation in children under study was FTT (91.4%), followed by developmental delay (79.3%) and dehydration episodes (72.4%). Likewise, FTT was the most frequent presenting feature in some previous studies.[1,18,23] Therefore, tubular disorders should be considered as an important differential diagnosis in all children with FTT. The high percentage of FTT may indicate delayed diagnosis. Indeed, we found that diagnosis after the first year of life is significantly associated with having FTT, which underscores the importance of early diagnosis. FTT in children with HRTD may be explained by multiple factors, including loss of nutrients, anorexia and vomiting, dehydration, chronic acidosis or alkalosis, chronic hypokalemia, and/or CKD.[4-6,8] As shown in our study, most children with FTT showed marked improvement in height and weight parameters in response to appropriate management, except for cases with Fanconi syndrome, which goes in line with previous studies.[4,18]

Nearly a third of children with HRTD in our study had nephrocalcinosis, Nephrocalcinosis is one of the common manifestations of HRTD, particularly dRTA and Bartter syndrome, which could be attributed to the alkaline urine and hypercalciuria, predisposing to calcium precipitation and stone formation.[22,24] Only one case (with cystinosis) developed CKD and started renal replacement therapy. Nephropathic cystinosis has been reported as the leading cause of CKD in previous studies.[1,18] The percentage of CKD in our study is quite lower than previous studies, such as 42% in Haffner et al.[21], 31% in Blázquez Gómez et al.[4], and 16. 2% in Al Mosawi.[22], which may be related to the shorter follow-up duration.

Patients under study were managed at PNU-SUH, which follows updated recommendations for management of HRTD. However, some patients had restricted access to certain medical services and medications due to economic constraints. Furthermore, other patients showed in-adherence to medications and irregular follow-up. The limited access to medication and patients' in-adherence to treatment coupled with delayed diagnosis represent major challenges to the proper management of children with HRTD in Egypt as well as other developing countries.[1,18,23]

The strengths of the present study include the first description of pediatric HRTD in Egypt, relying on characteristic criteria for diagnosis of different entities of HRTD, and the follow-up of children with HRTD for changes in weight and height z-scores. Nevertheless, our study has some limitations, first, it is a single-center study with relatively small sample size, which limit the generalizability of findings. However, HRTD are rare, and patients in this study were collected over seven years. Larger multicenter studies are recommended in areas with limited data on HRTD, including Egypt and other developing countries. Second, patients included in this study were referred to our pediatric nephrology center, and it is highly likely that other patients, particularly those with milder manifestations, were not recognized or referred by their physicians. Third, not all patients under study underwent comprehensive and repeated audiological assessment, which is important for early detection of hearing defects associated with certain HRTD. Last, diagnosis of HRTD relied on characteristic clinical and biochemical features, but without genetic confirmation. It would be important to study the molecular basis of HRTD among Egyptian children in future studies.

Over seven years, we identified 58 children with HRTD. The most common disorders were dRTA and Bartter syndrome, and the most common presenting manifestations were FTT, developmental delay, and dehydration episodes. Most children had improved growth with appropriate management and had preserved renal function. These data improve our knowledge on HRTD in southern Egypt and are crucial for increasing pediatrician awareness for appropriate management, which is essential for better outcome.

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**Ethical approval**

The present study was approved by the Medical Research Ethics Committee of Sohag Faculty of Medicine on April 11th, 2021 (IRB Registration Number: Soh-Med-21-04-04) and followed the ethical guidelines of the 1964 Declaration of Helsinki and its 2013 revision. Informed consent was obtained from parents or authorized legal guardians of children participated in this study.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: MMA, MK; data collection: MAMO, GABA, EA, MK; analysis and interpretation of results: MAMO, EA; draft manuscript preparation: MAMO, EA. All authors reviewed the results and approved the final version of the manuscript.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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**Original Article**

# Albuminuria is associated with 24-hour and night-time diastolic blood pressure in urinary tract infection with renal scarring

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## ABSTRACT

**Background.** We aimed to detect complications and associated risk factors in patients with renal scarring (RS) secondary to recurrent urinary tract infections (UTI).

**Methods.** Fifty patients with RS were compared with 25 patients without RS by means of, serum creatinine, 24-hour urinary creatinine clearance, and 24-hour urinary albumin levels. Office blood pressure (BP) examination and ambulatory BP monitoring (ABPM) were also performed.

**Results.** Vesicoureteral reflux was detected in 50 patients. Glomerular filtration rate (GFR) <90 ml/min/1.73 m2 was observed in 5 patients with RS but in no patient without RS. Albuminuria was significantly higher in patients with bilateral RS and severe RS. Patients with albuminuria had a significantly lower GFR than those without. All patients with ambulatory hypertension (HT) were in the RS group, and 60% of those had isolated nocturnal HT. Compared to those without RS, patients with RS had significantly higher SDS values for all BP readings, 24-hour and nighttime systolic and diastolic BP loads with significantly lower systolic dipping. GFR was negatively correlated with diastolic BP SDS and diastolic BP load in patients with RS. Daytime diastolic BP load was significantly higher in those with severe RS than in those with mild RS.

**Conclusions.** Isolated nocturnal HT could be an early sign of complications in RS of UTI. Albuminuria is related to increased BP and impaired renal function. Therefore, ABPM and assessing albuminuria should be a routine part of the follow-up. Diastolic BP elevations could be associated with worse outcomes in these patients.

**Key words:** albuminuria, ambulatory blood pressure monitoring, hypertension, renal scarring.

Urinary tract infection (UTI) is the most common bacterial infection in children after otitis media and is one of the major causes of acquired renal scarring (RS). Febrile UTIs associated with renal parenchymal inflammation may lead to nephron injury, resulting in permanent RS.[1]

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The incidence of developing permanent RS after UTI ranges from 15 to 60%, depending on factors such as age and sex of the patient, diagnostic criteria for UTI, reflux grade, and genetic susceptibility.[2]

Long-term complications of RS include hypertension (HT), proteinuria, impaired renal function, growth retardation, and problems during pregnancy.[3] However, the prevalence and onset of these problems are not fully understood, because they have an insidious onset and require long-term follow-up. Hypertension as a consequence of RS results in progressive renal dysfunction. Without early detection and timely control, HT accelerates the development

of end-stage kidney disease (ESKD). Therefore, close blood pressure (BP) monitoring is essential in patients with RS. Ambulatory blood pressure monitoring (ABPM) is superior to office BP measurement for detecting HT due to many factors such as obtaining multiple BP readings compared to a single measurement, eliminating BP measurement errors, and availability of daytime and nighttime BP measurements.[4] Some cases with RS have been diagnosed as hypertensive with ABPM, although office BP is normal.[5]

In the present study, we aimed to detect long-term complications of RS, identify the predictors of renal dysfunctions, evaluate the presence of HT by using ABPM and determine the effect of HT and albuminuria on progression.

## Material and Methods

### *Patients*

In this cross-sectional study, 75 patients who were older than five years of age and followed up for recurrent UTI at Hacettepe University Faculty of Medicine were included. Recurrent UTI was defined as two discrete febrile UTI episodes with positive urine cultures during a 12-month follow-up period. Patients with obstruction in the urinary tract, history of medication, and chronic disorders that could affect BP were excluded.

All patients that were regularly followed up were evaluated at the last follow-up visit. Age, sex, anthropometric, and office BP measurements were recorded. Height and body mass index (BMI) z-scores were calculated using the World Health Organization (WHO) Anthro-plus software. Serum creatinine, blood urea nitrogen (BUN), 24-hour urinary creatinine, and 24-hour urinary albumin levels were measured. Glomerular filtration rate (GFR) was calculated as creatinine clearance in 24-hour urine. Urinary albumin excretion greater than 30 mg/day was considered as albuminuria.

### *Blood pressure*

At the last visit, office BP measurement was performed for each patient using the auscultation method by a physician with the appropriate cuff size on the right arm after a minimum rest of 20 minutes. The results were evaluated according to the European Society of Hypertension guidelines.[6]

A 24-hour ABPM was performed with the AccuWin Pro v3 device (SunTech Medical, Inc., Morrisville, NC). The cuff of the device was placed on the non-dominant arm of the patient using an appropriately sized cuff. All patients were asked about their daily activities and sleep and awake periods, and these periods were recorded as daytime and nighttime. The devices were programmed to measure BP every 20 min during the daytime and every 30 min at nighttime. ABPM was standardized using the method of least median of squares, and BP percentiles were evaluated according to Wühl et al.'s reference values by patient sex and height.[7] Ambulatory HT was defined as a mean systolic and/or diastolic BP ≥95$^{th}$ percentile and systolic and/or diastolic BP load ≥25% for either the wake or sleep period of the study, or both. Ambulatory prehypertension was defined as a mean systolic and/or diastolic BP <95$^{th}$ percentile, but systolic and/or diastolic BP load ≥25% for either the wake or sleep period of the study, or both.[8] A blood pressure drop by at least 10% at night compared to daytime BP was defined as "dipping", and the absence of such a drop was defined as "non-dipping".

### *Imaging studies*

Vesicoureteral reflux (VUR) was assessed by voiding cystourethrography (VCUG) results. VUR was graded using 5 grades according to the International Reflux Study Standardization in Children.[9] The patients were grouped according to the VUR grade so that those with grade I and II VUR were in Group A, those with grade III in Group B, and those with grade IV and V in Group C. Patients with bilateral VUR, the grade was described as major reflux status.[10]

Diagnosis of RS was based on Tc-99m dimercaptosuccinic acid (DMSA) scintigraphy results, which were already available for the patients. Fifty patients had RS, and 25 patients did not have RS. Fifty patients with RS in two different scintigraphies at least 6 months apart were deemed to have permanent RS. All DMSA scintigraphies were re-evaluated by a nuclear medicine specialist by dividing the renal cortical area into 12 segments, with renal involvement categorized as mild scarring when 1-2 segments were affected, moderate scarring when 3-4 segments were affected, and severe scarring when more than 4 segments were affected. Patients with bilateral scarring were similarly categorized by the total scar area.[11,12]

### *Statistical analysis*

Statistical analyses were performed with SPSS version 24 (IBM Corp. Armonk, NY). Descriptive statistics included mean, standard deviation, minimum, maximum, median, and frequency values. Data distribution was tested using the Kolmogorov–Smirnov test. Quantitative data were analyzed using t-test and ANOVA test for parametric variables and Mann Whitney-U test and Kruskal-Wallis test for non-parametric variables. Qualitative variables were compared using the Chi-square test; when test assumptions were not met, Fisher's exact test was used. Correlation analyses were carried out using Pearson and Spearman correlation analyses. The study was approved by the Ethics Committee of Hacettepe University. Informed consent was obtained before enrollment.

## Results

### *Patient characteristics*

A total of 75 patients (63 females, 12 males) were enrolled in the study. Among them, 50 patients had RS, and 25 patients did not have RS. Among patients with RS, 27 (54%) patients had mild, 10 (20%) had moderate, and 13 (26%) had severe RS. Forty-four (88%) patients had unilateral RS, and six (12%) had bilateral RS. The mean duration from the first UTI to the last visit was 9.7±3.8 years for patients with RS and 8.2±3 years for those without RS (p=0.09). There was no significant difference between patients with and without RS regarding age, sex, duration of follow-up, height z-score, and BMI z-score (Table I).

Fifty (66.7%) patients had VUR, which was classified into three categories: groups A, B, and C. Thirty-four (68%) patients had unilateral VUR, and 16 (32%) had bilateral VUR. RS rate was higher in patients with bilateral VUR than patients with unilateral VUR, but it was not statistically significant (p=0.60). There was a correlation between VUR grade and RS severity (r=0.45, p=0.01) (Supplementary Table I).

### *Glomerular filtration rate at last visit*

At the last visit, in the RS group, the mean GFR was 123.7±33.0 ml/min/1.73 $m^2$, while in patients without RS, the mean GFR was 128.5±22.6 ml/min/1.73 $m^2$ (p=0.51). Similarly, GFR values did not significantly differ between patients with unilateral versus bilateral RS and between patients with mild, moderate, and

**Table I.** Demographic and clinical characteristics of the patients.

| Parameters | RS (+), n=50 | RS (-), n=25 | p |
|---|---|---|---|
| Sex (female/male), n/n | 41/9 | 22/3 | 0.50 |
| Duration of follow-up (years) | 9.7 ± 3.8 | 8.20 ± 2.95 | 0.09 |
| Age at last visit (years) | 12.6 ± 3.4 | 11 ± 3.46 | 0.06 |
| BMI-z score at last visit | 0.6 ± 1.2 | 0.5 ± 1.5 | 0.79 |
| Height z-score at last visit | 0.2 ± 1.1 | 0.6 ± 1.2 | 0.80 |

Data are presented as mean ± standard deviation
BMI: body mass index, RS: renal scarring

severe RS (p=0.32, p=0.50, respectively). The RS group included five patients with a GFR <90 ml/min/1.73 $m^2$, while the group without RS had no patient with a GFR <90 ml/min/1.73 $m^2$. In patients with RS, the mean duration of follow-up of the patients with GFR <90 ml/min/1.73 $m^2$ and >90 ml/min/1.73 $m^2$ was 13.06±1.71 years and 9.31±3.65 years, respectively (p=0.03).

### *Proteinuria*

At the last visit, 24-hour urinary albumin levels were measured in all patients except two patients with RS due to noncompliant issues. The median albumin excretion was 6.7 (IQR, 0.9-14.2) mg/day in the RS group and 5.5 (IQR, 3.4-8.0) mg/day in the non-RS group (p=0.40). In 24-hour urine, albuminuria was detected in seven (14.5%) patients with RS (six patients; 30-300 mg/day, one patient; >300 mg/day), while there was no patient with albuminuria in the non-RS group. The 24-hour albumin excretion was significantly higher in patients with severe RS than in patients with mild RS (p=0.04) (Fig. 1). Similarly, there was a correlation between 24-hour urinary albumin level and RS severity (Spearman correlation, r=0.334, p=0.02).

The median 24-hour urinary albumin was 4.1 (IQR, 0.0-12.1) mg/day in patients with unilateral RS and 27.1 (IQR, 10.6-108.8) mg/day in patients with bilateral RS (p=0.01). In patients with RS, 9.5% of patients with unilateral RS had albuminuria, while 50% of patients with bilateral RS had albuminuria. As a result, albuminuria incidence was significantly higher in patients with bilateral RS compared to patients with unilateral RS (p=0.01).

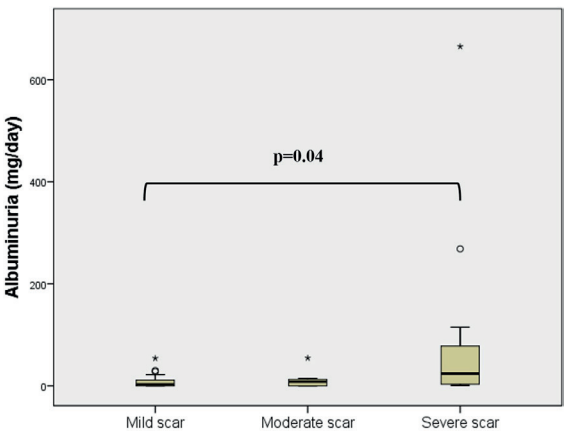


**Fig. 1.** Comparison of 24-hour urinary albumin level according to renal scarring severity.

The mean GFR of the patients with albuminuria and RS was 85.8 ± 37.1 ml/min/1.73 $m^2$, while the mean GFR of RS patients without albuminuria was 129.1 ± 28.7 ml/min/1.73 $m^2$ (p=0.001).

### *Blood pressure*

According to office BP measurements, among patients with RS, 9 (18%) out of 50 patients had high-normal BP, and 9 (18%) had HT. On the other hand, in the group without RS, HT was detected in only one patient (4%).

ABPM was performed in 73 patients (one patient with RS and the other without RS did not give informed consent). Of 55 patients with normal office BP measurements, 44 (80.4%) had normal ABPM, 9 had ambulatory prehypertension, and 2 had ambulatory hypertension (Supplementary Table II).

Ambulatory prehypertension was found in eight (16.3%) patients with RS and in four (16.6%) patients without RS. Ambulatory HT was found in 10 (20.4%) patients with RS. Ambulatory HT was not found in any patient without RS. Out of 10 patients with RS and ambulatory HT, six (60%) had isolated nocturnal BP elevation and normal daytime BP readings, while four (40%) had daytime and nighttime HT. Similarly, six (75%) of eight patients with ambulatory prehypertension and RS had an isolated nocturnal BP load ≥25% with daytime BP load <25% and two of them had daytime and nighttime blood pressure load ≥25%.

According to ABPM results, 24-hour systolic BP standard deviation scores (SDS), 24-hour diastolic BP SDS, daytime systolic BP SDS, daytime diastolic BP SDS, nighttime systolic BP SDS, nighttime diastolic BP SDS, 24-hour MAP SDS, daytime MAP SDS, and nighttime MAP SDS values were significantly higher in patients with RS than in those without. 24-hour systolic

**Table II.** Comparison of blood pressure standard deviation scores, blood pressure load, and dipping values in patients with and without renal scarring.

| Parameters | RS (-), n=24 | RS (+), n=49 | p |
|---|---|---|---|
| 24-hour SBP SDS† | -0.92 ± 1.15 | -0.01 ± 1.25 | 0.03 |
| 24-hour DBP SDS‡ | -1.39 (-3.66-0.90) | -0.84 (-3.01-3.71) | 0.02 |
| 24-hour MAP SDS† | -0.90 ± 0.98 | -0.15 ± 1.25 | 0.01 |
| Daytime SBP SDS† | -0.94 ± 1.09 | -0.26 ± 1.23 | 0.02 |
| Daytime DBP SDS‡ | -1.67 (-3.36-0.93) | -1.29 (-2.53-3.06) | 0.03 |
| Daytime MAP SDS‡ | -1.16 (-2.85-0.79) | -0.74 (-1.90-3.55) | 0.02 |
| Nighttime SBP SDS† | -0.40 ± 0.91 | 0.59 ± 1.06 | <0.001 |
| Nighttime DBP SDS† | -0.38 ± 0.84 | 0.32 ± 1.06 | 0.01 |
| Nighttime MAP SDS† | -0.32 ± 0.78 | 0.49 ± 1.10 | 0.002 |
| 24-hour SBP load (%)‡ | 6.5 (0-21) | 10 (0-82) | 0.02 |
| 24-hour DBP load (%)‡ | 2.5 (0-21) | 6 (0-90) | 0.02 |
| Daytime SBP load (%)‡ | 6.5 (0-23) | 8 (0-77) | 0.17 |
| Daytime DBP load (%)‡ | 1 (0-28) | 4 (0-86.7) | 0.13 |
| Nighttime SBP load (%)‡ | 0 (0-29) | 17 (0-92) | <0.001 |
| Nighttime DBP load (%)‡ | 0 (0-30) | 11.1 (0-100) | 0.002 |
| Systolic dipping (%)† | 10.86 ± 3.98 | 8.27 ± 3.98 | 0.01 |
| Diastolic dipping (%)† | 15.88 ± 6.33 | 13.79 ± 6.30 | 0.19 |

†Mean ± standard deviation, ‡median (range).
SBP: systolic blood pressure, DBP: diastolic blood pressure, MAP: mean arterial pressure, RS: renal scarring, SDS: standard deviation score

BP load, 24-hour diastolic BP load, nighttime systolic BP load, and nighttime diastolic BP load were significantly higher in patients with RS than in those without. Furthermore, patients with RS had significantly lower systolic BP dipping than patients without RS (Table II). In addition, when the difference between daytime and nighttime blood pressure SDS and blood pressure loads in patients with RS was evaluated; nighttime systolic blood pressure SDS was significantly higher than daytime systolic blood pressure SDS, nighttime diastolic blood pressure SDS was significantly higher than daytime diastolic blood pressure SDS, and nighttime MAP SDS was significantly higher than daytime MAP SDS (p=<0.001, p=<0.001, p=<0.001 respectively). Similarly, nighttime SBP load was significantly higher than daytime SBP load and nighttime diastolic BP load was significantly higher than daytime diastolic BP load in patients with RS (p=<0.001, p=<0.001 respectively).

According to RS severity, daytime diastolic BP load was significantly higher in the severe RS group than in the mild RS group (median: 7.5; range: 0.0-86.7 versus median: 3.0; range: 0.0-46.8, p=0.01). Other BP loads, BP SDS values, and dipping values were not significantly different according to RS severity.

Among patients with RS, 24-hour diastolic BP SDS and nighttime diastolic BP SDS values were significantly higher in patients with albuminuria than in those without (Table III) (p=0.035, p=0.02).

A significant negative correlation was found between GFR and 24-hour diastolic BP SDS (p=0.005, r=-0.47), nighttime diastolic BP SDS (p= 0.036, r=-0.34), 24-hour MAP SDS (p= 0.033, r=-0.28), 24-hour diastolic BP load (p= 0.020, r=-0.32), and nighttime diastolic BP load (p= 0.030, r=-0.29) in patients with RS (Fig.2).

**Table III.** Comparison of blood pressure standard deviation scores of patients with and without albuminuria among patients with renal scar.

| Variables | Blood pressure | Albuminuria <30 mg/day, n=40 | Albuminuria >30 mg/day, n=7 | p |
|---|---|---|---|---|
| 24-hour | Systolic SDS[a] | -0.02 ± 1.19 | -0.27 ± 1.59 | 0.62 |
| | Diastolic SDS[b] | -1.04 (-3.01/1.83) | -0.62 (-0.84/3.71) | 0.04 |
| | MAP SDS[b] | -0.37 (-2.18/2.57) | -0.29 (-1.02/4.47) | 0.49 |
| Daytime | Systolic SDS[a] | -0.25 ± 1.18 | -0.59 ± 1.57 | 0.50 |
| | Diastolic SDS[b] | -1.44 (-2.5/1.80) | -0.60 (-1.69/3.06) | 0.11 |
| | MAP SDS[b] | -0.80 (-1.9/2.50) | -0.47 (-1.66/3.55) | 0.63 |
| Nighttime | Systolic SDS[a] | 0.54 ±1.04 | 0.57 ± 1.21 | 0.93 |
| | Diastolic SDS[b] | 0.10 ± 0.94 | 1.12 ± 1.28 | 0.02 |
| | MAP SDS[a] | 0.32 ± 0.95 | 0.98 ± 1.50 | 0.13 |

[a]Mean ± standard deviation, [b]median (range). MAP: mean arterial pressure, SDS: standard deviation score.

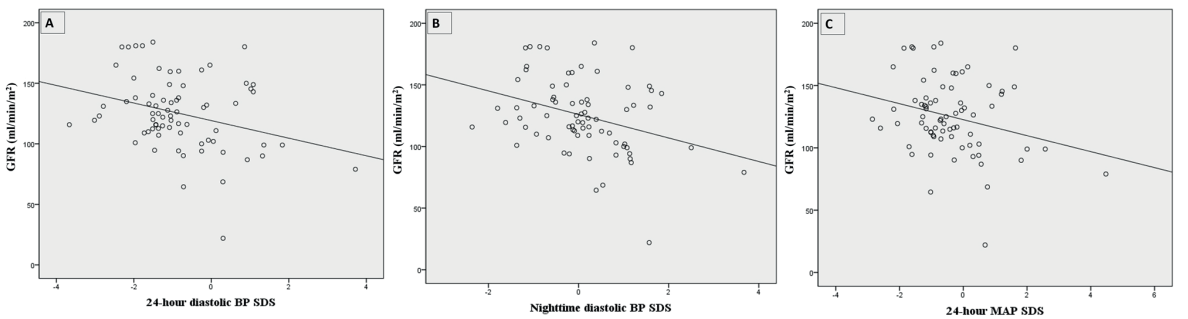


**Fig. 2.** Correlation between glomerular filtration rate (GFR) and (A) 24-hour diastolic blood pressure (BP) standard deviation score (SDS), (B) nighttime diastolic BP SDS, (C) 24-hour mean arterial pressure (MAP) SDS among patients with renal scarring (p=0.005, 0.036, 0.033, r= -0.37, -0.34, -0.28, respectively).

## Discussion

In this study, we investigated long-term complications of RS in a pediatric cohort. 24-hour urinary albumin level was higher in patients with severe and bilateral RS. In addition, the GFR of patients with albuminuria was significantly lower than those without. These results suggest that albuminuria is a risk factor for progression. Furthermore, in patients with RS, 6 out of 10 patients with ambulatory hypertension and, 6 out of 8 patients with ambulatory prehypertension had isolated elevated nocturnal blood pressure. The fact that this situation can only be detected with ABPM highlights the importance of using ABPM in routine care. Another important finding of this study is GFR was negatively correlated with diastolic BP in patients with RS. These results suggest that diastolic blood pressure elevation and isolated nocturnal blood pressure elevation may be early signs of sustained hypertension and progression.

The incidence of chronic kidney disease (CKD) and ESKD is highly variable among patients with RS secondary to pyelonephritis. Gebäck et al.[13] examined 86 patients with a median age of 41 years who had UTI in childhood and reported one patient with stage 3 CKD, 14 patients with stage 2 CKD, and 43 patients with stage 1 CKD and that patients with bilateral RS had significantly lower GFR. Patzer et al.[14] examined 61 patients aged 5-18 years with a history of recurrent febrile UTI and RS, without obstructive uropathies. They found that nine

patients had GFR <90 ml/min/1.73 $m^2$, and two had GFR ≤50 ml/min/1.73 $m^2$. Our study detected five patients with RS who had a GFR lower than 90 ml/min/1.73 $m^2$. The duration of follow-up of the patients with GFR <90 ml/min/1.73 $m^2$ was longer than those with GFR >90 ml/min/1.73 $m^2$. This shows the detrimental effect of RS on GFR, especially over time.

Despite the relationship between HT and RS being well known, its incidence and risk factors are not clear. Although the likelihood of RS causing HT is better understood in adulthood, there is a need for additional studies on its incidence and time of onset in children.[15] Although office BP measurement is easy and cheap, ABPM was shown to be more specific for the diagnosis of HT.[16] Fidan et al.[17] monitored 240 patients with VUR and a mean age of 7.1 years for 24 months. They found that although office BP was normal, 17 patients were diagnosed with HT using ABPM. Patzer et al.[14] studied 61 patients with RS secondary to UTI and diagnosed HT in 17 (28%) patients using ABPM and in 24 (39%) patients using office BP measurements. In our study, 49 patients with RS underwent ABPM, ten (20.4%) of these patients had ambulatory HT, and eight (16.3%) had ambulatory prehypertension. Seven of the patients with RS had no HT on office BP, three had ambulatory HT, and four had ambulatory prehypertension with ABPM. These results indicate that in patients with RS, ABPM should be a part of routine care, especially for the detection of masked hypertension.

Furthermore, in patients with RS, six out of ten (60%) patients with ambulatory HT had isolated nocturnal HT, while of six out of eight (75%) patients with ambulatory prehypertension had isolated nocturnal BP elevation, indicating that these patients have a high rate of nocturnal BP elevation. When daytime and nighttime blood pressure SDS and blood pressure loads were compared in patients with RS, nighttime values were found to be significantly higher than daytime. Blood pressure varies according to the circadian rhythm, which is associated with the sympathetic nervous system and renin-angiotensin systems. Previous studies have shown that nocturnal hypertension is associated with end-organ injury and poor prognosis in patients with diabetes, CKD, and organ transplant recipients in adults and children.[18-24] Data from the Cardiovascular Comorbidity in Children with Chronic Kidney Disease Study (4 C) established significant associations between nighttime HT and left ventricular hypertrophy, elevated cIMT and elevated pulse wave velocity.[25] Besides all these, there are few data about nocturnal hypertension in patients with RS.[14] According to our data, HT in children with RS is mostly seen as isolated nocturnal HT. Because isolated nocturnal HT may be an early finding of sustained HT, early detection and treatment of this situation would be vital in terms of good prognosis.

In adults, the relationship between microalbuminuria and progressive renal failure and cardiovascular risk is well known.[26-28] However, there are a few studies on this topic in children. Although hyperfiltration in the remaining nephrons and glomerulosclerosis is thought to cause microalbuminuria in patients with RS, microalbuminuria itself also has nephrotoxic effects. Studies in children with RS have shown that microalbuminuria is associated with lower GFR and severe and bilateral RS.[29,30] Our study also demonstrated that 24-hour albumin excretion was significantly higher in patients with severe RS and bilateral RS. Patients with RS and albuminuria had lower GFR and higher BP readings than that patients without albuminuria. These results indicated that patients with albuminuria were in the high-risk group, had worse ABPM readings, and lower renal function, suggesting that albuminuria should be sought in the routine follow-up of these patients. When it is detected, they should be considered in the risk group for BP elevation and impaired renal function.

Diastolic hypertension is not a well-known entity. However, several studies have found that diastolic BP elevations in childhood are associated with secondary HT.[31,32] Studies have revealed that isolated diastolic HT is

rare but independently affects the risk of adverse cardiovascular events in adults.[33,34] Conversely, isolated diastolic HT was not found to be significantly associated with increased cardiovascular risk in another study.[35] There is insufficient data on the importance of diastolic HT in patients with RS. In this study, a higher diastolic BP was found in the risk group with albuminuria and low GFR, suggesting that diastolic BP elevations may be an early sign of sustained HT and poor prognoses.

There are several limitations in our study, such as the relatively small number of patients and the inability to compare ABPM results with end-organ damage findings such as cIMT and left ventricular hypertrophy. On the other hand, the relatively long follow-up period and the evaluation of BP with ABPM are the strengths of the study.

In conclusion, early detection of RS-associated HT and microalbuminuria is a crucial step for preventing progression to renal failure. There was a negative correlation between GFR and diastolic BP in patients with RS. Isolated nocturnal BP elevation may be one of the earliest signs during the follow-up of these patients. Therefore, these patients should be evaluated by ABPM intermittently.

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**Ethical approval**

Approval was obtained from the Non-Interventional Clinical Researches Ethics Board of Hacettepe University (28th February 2017, GO 17/94-08). The procedures used in this study adhere to the tenets of the Declaration of Helsinki. Informed consent was obtained from all individual participants included in the study.

**Author contribution**

The authors confirm contribution to the paper as follows: All authors contributed to the study conception and design. Data collection and analysis: DB and RT, draft manuscript preparation: DB; and all authors commented on previous versions of the manuscript and approved the final manuscript.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

**Supplementary information is available at:**

http://www.turkishjournalpediatrics.org/uploads/turkjped.2022.766.S1.pdf

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**Original Article**

# Assessing fatigue and related factors in adolescents with familial Mediterranean fever (FMF): psychometric properties of the PedsQL Multidimensional Fatigue Scale

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## ABSTRACT

**Background.** Fatigue is a common problem in pediatric rheumatic diseases and is associated with poor quality of life. However, no validated methods are available to measure fatigue in adolescents with familial Mediterranean fever (FMF). The aim of the study was to establish validity and reliability for the child self-report PedsQL Multidimensional Fatigue Scale (PedsQL-MFS) and to investigate the effects of physical characteristics, disease-related characteristics, sleep quality/duration, and the amount of physical activity on fatigue in adolescents with FMF.

**Methods.** Seventy-one adolescents with FMF (13-18 years) were included. Children were examined regarding physical- and disease-related characteristics and completed patient-reported outcome measures (PROMs) regarding sleep quality/duration, physical activity levels, and fatigue. PedsQL-MFS was re-completed within the following 7-14 days.

**Results.** PedsQL-MFS demonstrated excellent test-retest reliability (ICC in 95% CI: 0.877-0.958) and internal consistency (Cronbach's $\alpha$: 0.928). All items contributed to the total score (item-total correlation >0.3). PedsQL-MFS scores were significantly correlated to fatigue (r: -0.666, p<0.001), physical activity (r: 0.373, p<0.001), sleep quality ($r_s$: 0.678, p<0.001), and sleep duration (r: 0.473, p<0.001). Being female, having attacks in the last six months, a sleep duration of less than seven hours, and engaging in less physical activity resulted in higher fatigue.

**Conclusions.** PedsQL-MFS seems to be feasible for assessing fatigue in adolescents with FMF. Sex, recent attacks, sleep, and physical activity should be taken into consideration in the fatigue management of patients with FMF.

**Key words:** Fatigue, child, familial Mediterranean fever, sleep, physical activity.

Familial Mediterranean fever (FMF) is the most prevalent monogenic auto-inflammatory disease.[1,2] It is especiailly common in people living in/originating from the Mediterranean basin and the Middle East.[1,2] However, the incidence of FMF is on the rise all over the world as a result of migrations, and FMF is being diagnosed more frequently in regions where the disease was thought to be uncommon such as Europe, Japan, and United States.[3-5] The disease onset is usually during childhood and progresses with recurrent attacks characterized by fever, peritonitis, pleuritis, arthritis/arthralgia, or erysipelas-like skin findings.[1,2] Patients are usually considered symptom-

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free between attacks. However, subclinical inflammation and chronic disease course may have a negative impact on the quality of life even between attacks.[6-8]

Fatigue is defined as a persistent feeling of exhaustion and lack of energy which can originate from physical, mental/emotional, sleep-related, and disease-related factors.[9-11] Moderate to severe fatigue is reported in pediatric and adult patients with different chronic conditions including rheumatic diseases and is a debilitating factor that affects many aspects of daily living and physical functioning.[10,12-14] Concurringly, European Alliance of Associations for Rheumatology (EULAR) and American College of Rheumatology (ACR) recommend assessing fatigue as a part of regular follow-up in rheumatic and musculoskeletal diseases.[15,16] To the best of our knowledge, solely Ozdel et al.[17] attempted to evaluate fatigue with an unvalidated questionnaire previously and reported that children with FMF presented higher fatigue perception than their healthy peers. However, a more comprehensive evaluation of fatigue using valid and reliable tools may help to better understand the impact of fatigue in children with FMF.

The PedsQL Multidimensional Fatigue Scale (PedsQL-MFS) is a self-report outcome measure, developed to assess fatigue in children of different age groups.[18] The psychometric properties of PedsQL-MFS were demonstrated to be adequate in various conditions such as cancer, obesity, juvenile idiopathic arthritis, juvenile systemic lupus erythematosus, and juvenile fibromyalgia.[18-20]

This study aimed to (a) investigate the psychometric properties of PedsQL-MFS, and (b) compare fatigue according to sex, disease-related factors, sleep duration, and physical activity in adolescents with FMF.

## Material and Methods

This was a cross-sectional validation study. The ethical approval was obtained from the Non-Interventional Clinical Studies Institutional Review Board of İzmir Katip Çelebi University (date: 24.06.2021, number: 0308). All procedures were performed in accordance with the Declaration of Helsinki. Adolescents and their parents signed a written informed consent prior to their participation.

### *Participants*

G*Power 3.1.9.4 software was used to calculate the sample size. An expected minimum correlation coefficient of 0.3, a minimum type I error of 0.05, and a maximum type II error of 20% revealed that at least 64 participants were necessary to explore the convergent validity of PedsQL-MFS.

Adolescents (aged 13-18 years) diagnosed with FMF based on Turkish pediatric FMF criteria[21] were included in the study between July 2021 and July 2022. Exclusion criteria were (a) concomitant conditions such as fibromyalgia or other systemic diseases that cause fatigue, (b) intra-articular injection and/or surgery in the last six months.

### *Procedures*

Adolescents who met the eligibility criteria were invited to participate. Physical characteristics (age, sex, body-mass index), and clinical parameters (*MEFV* mutation type, time since the onset of the symptoms, time since diagnosis, the dosage of colchicine and/or other medication, and the number of FMF attacks in the last six months) were recorded on a structured form. Subsequently, adolescents filled out the PedsQL-MFS child self-report[20], The Checklist Individual Strength (CIS)[22], Sleep Quality Scale and Sleep Variables Questionnaire (SQS-SVQ)[23], and Children's Leisure Assessment Scale (CLASS).[24] All assessments were

completed within the same day. All adolescents re-completed PedsQL-MFS within the following 7-14 days.

### *Outcome measures*

*The PedsQL Multidimensional Fatigue Scale (PedsQL-MFS)*: The Turkish translation of PedsQL-MFS, along with other languages was provided by the original author Varni under the license of Mapi Research Trust Organization. Permission to use the Turkish version of PedsQL-MFS was obtained from Mapi Research Trust Organization prior to the study. PedsQL-MFS has separate forms for different age categories. PedsQL-MFS for children between 13-18 years of age were administered to adolescents (child self-report) in the present study.[20] PedsQL-MFS included 18 identical items under three sub-categories: (1) General Fatigue, (2) Sleep/ Rest Fatigue, and (3) Cognitive Fatigue. Each sub-category included six items, and each item was scored on a 5-point Likert scale between zero (the absence of a problem in during the past month) and four (the problem was almost always present during the past month). The item scores were reversed and were linearly transformed into a score between 0 and 100 (0=100, 1=75, 2=50, 3=25, 4=0). The total score and sub-category scores were calculated by summing up item scores and dividing the outcome by the number of items. The final score ranged between 0-100 with higher scores indicating less fatigue.

*The Checklist Individual Strength (CIS)*: CIS was employed to examine the convergent validity of the PedsQL. CIS was used to assess fatigue in children with FMF previously.[17] CIS scores were investigated in four sub-categories: (1) Subjective Fatigue (eight items), (2) Concentration (five items), (3) Motivation (four items), and (4) Physical Activity (three items). CIS consisted of 20 fatigue-related items (e.g., "*I feel tired*") scored on a 7-point Likert scale (between 1: "yes, it is true", and 7: "no, it is false"). Eleven out of the 20 items were reverse scored. The total score and sub-category scores were calculated by summing up the scores. Higher scores indicated a higher perception of fatigue.[22]

*Sleep Quality Scale and Sleep Variables Questionnaire (SQS-SVQ)*: SQS-SVQ included a total of 15 items and was used to measure the quality of sleep (0-21, higher scores indicating better sleep quality) and total time spent sleeping (in minutes) in the present study. The calculations for each component were performed following the instructions by Onder et al.[23]

*Children's Leisure Assessment Scale (CLASS):* CLASS was used to determine the level of physical activity.[24] CLASS consisted of 45 items of which 31 questions physical activities (e.g., swimming, dancing, walking, etc.) and 14 questions sedentary behaviors (e.g., watching TV, listening to music, etc.). Responders indicated "yes" or "no" representing whether they performed the relevant activity on a typical week. Children who marked "yes" for a particular item were also inquired regarding frequency and total time (in minutes or hours). The option "other" was provided for activities/ behaviors which were not included in the questionnaire but were performed by children. The energy expenditure for each physical activity was calculated using the formula: *(METs of an activity)*(minutes spent in doing the activity in a week)* with the metabolic equivalent (MET) values provided by Ainsworth et al.[25] The total physical activity was computed by summing up the METs calculated for each item. Higher MET values indicated a higher level of physical activity.

### *Statistical analysis*

Statistical analyses were performed using IBM SPSS Statistics for Windows, version 22.0 (Armonk, NY, USA). The normality of the data was analyzed by examining the Kolmogorov-

Smirnov test, histograms, detrended Q-Q plots, and skewness/kurtosis values. Mean and standard deviations or median and interquartile ranges were used depending on the distribution of the data. Categoric variables were expressed as numbers (n) and percentages (%). The missing data was handled as suggested by Varni et al.[18] $p<0.05$ was considered statistically significant.

*Reliability:* The reliability of PedsQL-MFS was explored by analyzing corrected item-total correlation, and internal consistency (Cronbach's $\alpha$). A change of less than 10% in internal consistency with the exclusion of items, and a correlation coefficient higher than 0.3 in item-total correlation were assumed to keep any individual item. Intraclass Correlation Coefficients 2,1 (ICC) were calculated to establish test-retest reliability.

*Validity:* Convergent validity of PedsQL-MFS was investigated by calculating correlation coefficients between PedsQL-MFS and CIS, CLASS, and SQS-SVQ results. Pearson (r) and Spearman ($r_s$) correlation coefficients were calculated for normally and non-normally distributed variables, respectively. Obtained correlations were interpreted as follows: negligible=0 to 0.29, poor=0.30 to 0.49, moderate=0.50 to 0.69, good=0.70 to 0.89, and excellent=0.90 to 1.00.[26]

*Sub-group comparisons:* Mann-Whitney U test was employed to compare fatigue among different sub-groups according to physical characteristics (sex), disease-related characteristics (number of attacks, colchicine use, presence of M694V mutation), and other variables (sleep duration, level of physical activity). Cohen's d (*d)* values were calculated for estimating effect size.[27] Effect sizes were interpreted as follows: $d<0.2$=negligible, $0.2\leq d<0.5$=small, $0.5\leq d<0.8$=moderate, $0.8\leq d<1.3$=large, $1.3\leq d$=very large. Seven hours of sleep and 2560 MET-min/week of physical activity were used as cut-off values to divide patients into sub-groups. Seven hours of sleep was recommended by Leger et al.[28] and 2560 MET-min/week was calculated using the equation *60 minutes*7 days*6 MET=2560* based on the recommendation of at least 60 minutes of moderate to vigorous activity (4-8 METs) every day for individuals under the age of 18 years by the World Health Organization.[29]

## Results

The study was completed with the participation of 71 adolescents with FMF. Re-test data was obtained from 51 adolescents. The number of missing items was detected as minimal (0.5%). The results regarding physical characteristics, disease-related characteristics, and PROMs were presented in Table I.

The internal consistency of PedsQL-MFS was excellent (Cronbach's $\alpha$: 0.928). No significant change was observed in internal consistency with the exclusion of any item (change <10%), and all the items contributed to the total score (item-total correlation >0.3), (Table II). The test-retest reliability was determined as good to excellent (ICCs: between 0.881 and 0.928, $p<0.05$) for total- and sub-scores (Table III).

Significant poor to good correlations were observed between PedsQL-MFS and CIS total score (r: -0.666, $p<0.001$), physical activity levels (r: 0.373, $p<0.001$), sleep quality ($r_s$: 0.628, $p<0.001$), and sleep duration ($r_s$: 0.473, $p<0.001$) indicating the convergent validity of PedsQL-MFS (Table IV).

PedsQL-MFS scores were lower; in females ($d$=0.77, $p<0.05$), in children who had attacks in the last six months ($d$=0.85, $p<0.05$), in children who were sleeping less than seven hours a day ($d$=0.94, $p<0.001$), and in children who engaged in less than 2560 MET-min/week of physical activity ($d$=0.59, $p<0.05$), (Table V). No between-group differences were detected in PedsQL-MFS scores according to colchicine use or the presence of M694V mutation ($p>0.05$), (Table V).

**Table I.** Demographic and disease-related characteristics of children with Familial Mediterranean Fever.

| | Median (Q1-Q3) or n (%) (n=71) |
|---|---|
| Sex (Female/Male), n | 34/37 |
| Age, yr | 15 (14-16) |
| BMI (kg/m$^2$) | 20.11 (18.65-23.48) |
| Time since the onset of symptoms, yr | 10 (7-14) |
| Time since the diagnosis, yr | 9 (6-11) |
| Number of attacks in last six months, n | 1 (0-3) |
| Colchicine | |
| Not using, n (%) | 12 (16.9%) |
| 0.5 mg/day, n (%) | 8 (11.3%) |
| 1 mg/day, n (%) | 23 (32.4%) |
| 1.5 mg/day, n (%) | 18 (25.4%) |
| 2 mg/day, n (%) | 10 (14.1%) |
| *MEFV* mutations | |
| Undefined | 21 (29.6%) |
| M694V Homozygous | 15 (21.1%) |
| M694V Heterozygous | 13 (18.3%) |
| R202Q Homozygous | 3 (4.2%) |
| E148Q Homozygous | 1 (1.4%) |
| E148Q Heterozygous | 1 (1.4%) |
| P369S Heterozygous | 1 (1.4%) |
| E148Q/V726A Heterozygous (Compound) | 1 (1.4%) |
| E148Q/M964M Heterozygous (Compound) | 1 (1.4%) |
| E148Q/R202Q Heterozygous (Compound) | 3 (4.2%) |
| K695R/R202Q Heterozygous (Compound) | 1 (1.4%) |
| M6801/M694V Heterozygous (Compound) | 1 (1.4%) |
| M694V/R202Q Heterozygous (Compound) | 3 (4.2%) |
| M694V/R202Q Homozygous (Compound) | 1 (1.4%) |
| M694V Heterozygous/R202Q Homozygous (Compound) | 1 (1.4%) |
| M694V/E148Q Heterozygous (Compound) | 2 (2.8%) |
| M694V/M6801 Heterozygous (Compound) | 1 (1.4%) |
| M696V/R202Q Homozygous (Compound) | 1 (1.4%) |
| CIS | |
| Subjective fatigue (score: 0-56) | 27 (20-35) |
| Concentration (score: 0-35) | 18 (12-22) |
| Motivation (score: 0-28) | 15 (10-18) |
| Activity (score: 0-21) | 10 (7-13) |
| Total (score: 0-140) | 73 (54-83) |
| CLASS | |
| Total physical activity (METs min/week) (n=61) | 2585 (986-5155) |
| SQS-SVQ | |
| Sleep quality (score: 0-21) | 15 (13-17) |
| Sleep duration (min/day) | 466 (405-510) |

CIS: The Checklist Individual Strength, CLASS: Children's Leisure Assessment Scale, SQS-SVQ: Sleep Quality Scale and Sleep Variables Questionnaire, MET: metabolic equivalent, Q1-Q3: first and third quartiles.

**Table II.** Item-Total Correlations of PedsQL-MFS.

| Item | Corrected Item-Total Correlation | Cronbach's Alpha if Item Deleted |
|---|---|---|
| General Fatigue 1 | 0.707 | 0.922 |
| General Fatigue 2 | 0.620 | 0.924 |
| General Fatigue 3 | 0.757 | 0.921 |
| General Fatigue 4 | 0.623 | 0.924 |
| General Fatigue 5 | 0.703 | 0.922 |
| General Fatigue 6 | 0.746 | 0.921 |
| Sleep/Rest Fatigue 1 | 0.489 | 0.927 |
| Sleep/Rest Fatigue 2 | 0.586 | 0.925 |
| Sleep/Rest Fatigue 3 | 0.618 | 0.924 |
| Sleep/Rest Fatigue 4 | 0.338 | 0.930 |
| Sleep/Rest Fatigue 5 | 0.529 | 0.926 |
| Sleep/Rest Fatigue 6 | 0.553 | 0.926 |
| Cognitive Fatigue 1 | 0.741 | 0.921 |
| Cognitive Fatigue 2 | 0.608 | 0.924 |
| Cognitive Fatigue 3 | 0.697 | 0.922 |
| Cognitive Fatigue 4 | 0.479 | 0.927 |
| Cognitive Fatigue 5 | 0.728 | 0.922 |
| Cognitive Fatigue 6 | 0.670 | 0.923 |
| | | Total Cronbach's alpha= 0.928 |

**Table III.** Test-retest reliability of PedsQL-MFS.

| | Test (Mean±SD) (n=51) | Re-test (Mean±SD) (n=51) | ICC (95% CI) |
|---|---|---|---|
| Child self-report | | | |
| General Fatigue | 60.87±26.72 | 60.86±24.21 | 0.909 (0.846-0.947) |
| Sleep/Rest Fatigue | 56.51±21.65 | 57.43±22.53 | 0.899 (0.829-0.941) |
| Cognitive Fatigue | 68.89±23.84 | 71.24±22.50 | 0.881 (0.801-0.930) |
| Total | 62.09±20.89 | 63.18±20.09 | 0.928 (0.877-0.958) |

CI: confidence interval, ICC: Intraclass correlation coefficient, SD: standard deviation.

**Table IV.** Convergent validity of PedsQL-MFS.

| n=71 | PedsQL-MFS | | | |
|---|---|---|---|---|
| | General | Sleep/Rest | Cognitive | Total |
| CIS | | | | |
| Subjective fatigue | -0.719**# | -0.546**# | -0.414**# | -0.652**# |
| Concentration | -0.528**# | -0.210# | -0.555**# | -0.540**# |
| Motivation | -0.517**# | -0.356*# | -0.292*# | -0.465**# |
| Activity | -0.459**# | -0.292*# | -0.320*# | -0.418**# |
| Total | -0.714**† | -0.483**† | -0.511**† | -0.666**† |
| CLASS | | | | |
| Total physical activity (METs min/week) | 0.397**† | 0.208† | 0.324*† | 0.373**† |
| SQS-SVQ | | | | |
| Sleep quality (score: 0-21) | 0.647**# | 0.555**# | 0.429**# | 0.628**# |
| Sleep duration (min/day) | 0.438**† | 0.513**† | 0.287† | 0.473**† |

CIS: The Checklist Individual Strength, CLASS: Children's Leisure Assessment Scale, SQS-SVQ: Sleep Quality Scale and Sleep Variables Questionnaire, mins: minutes

#: Spearman's correlation coefficient

†: Pearson's correlation coefficient

*: p<0.05, **: p<0.001

**Table V.** Comparison of fatigue according to demographic, disease-related or physical characteristics.

| | | PedsQL-MFS Median (Q1-Q3) | *p** | *d* |
|---|---|---|---|---|
| Sex | Female (n= 34) | 56.9 (38.9-68.4) | *0.002* | 0.77 |
| | Male (n= 37) | 69.4 (59.0-79.9) | | |
| Attacks in last six months | 0 (n= 33) | 75.0 (53.8-83.3) | *0.004* | 0.85 |
| | ≥1 (n= 38) | 63.2 (43.4-67.0) | | |
| Colchicine | Not using (n=12) | 64.6 (43.4-79.1) | *0.836* | - |
| | Using (n=59) | 65.7 (46.5-76.7) | | |
| M694V mutation | Present (n=38) | 66.3 (41.3-74.7) | *0.331* | - |
| | Not present (n=12) | 64.6 (49.7-87.5) | | |
| Sleep duration | <7 hours (n=21) | 44.4 (30.5-76.3) | *<0.001* | 0.94 |
| | ≥7 hours (n=48) | 68.1 (53.5-82.6) | | |
| Total physical activity | ≥2560 MET-min/week (n=31) | 67.8 (54.2-77.8) | *0.025* | 0.59 |
| | <2560 MET-min/week (n=30) | 53.5 (38.9-75.3) | | |

MET: metabolic equivalent, min: minute, d: Cohen's *d* (effect size), Q1-Q3: first and third quartiles.
*: Mann-Whitney U test

## Discussion

The present study was the first to present the psychometric properties of PedsQL-MFS in children with FMF. PedsQL-MFS demonstrated good validity and reliability. Being female, having attacks in the last six months, sleeping less than seven hours, and engaging in less physical activity had a negative impact on fatigue. On the other hand, the presence of M694V mutation or colchicine usage showed no significant impact on fatigue.

The rates of missing data were at an acceptable level and similar to previously reported values[20] indicating PedsQL-MFS is feasible in children with FMF. The ICC values were in general agreement with the results of previous studies that investigated the reliability of PedsQL-MFS in children with rheumatic diseases.[20] As there were no validated gold standard tools to evaluate fatigue in children with FMF, we could not examine the concurrent validity of PedsQL-MFS. Additionally, the ability to discriminate children with FMF from healthy children in terms of fatigue or sensitivity/specificity in detecting patients with and without fatigue were not investigated due to absence of a control group and a relatively small sample size. Thus, these psychometric properties of PedsQL-MFS are yet to be investigated in the future.

Fatigue has been inadequately investigated in children with FMF. Best to our knowledge, only the study by Ozdel et al.[17] reported higher fatigue among children with FMF compared to healthy peers. Additionally, they mentioned that children with higher colchicine dosage and older age presented more severe fatigue, while sex or attack frequency had no effect on the level of fatigue. The differences between our studies may be related to preferred PROMs for capturing fatigue. Ozdel et al.[17] employed CIS to evaluate the fatigue, however, the utility of CIS in patients with FMF is unknown as no validation study exists. On the other hand, we demonstrated good to excellent validity and reliability regarding PedsOL-MFS in the present study. Additionally, groups were more heterogeneous in terms of sex distribution and presence of attack frequency in the study by Ozdel et al.[17] compared to the present study. Similar to our results, Duruoz et al.[14] also showed that sex and previous attacks might affect fatigue, and colchicine usage have no impact on fatigue in adult patients with FMF.

Sleep is an important determinant of the quality of life and sleep-related problems are common in children with chronic diseases.[30,31] Poorer sleep quality was reported in children with FMF previously, and sleep disturbances were found to be associated with pain, anxiety, and the number of attacks.[31] The current study was the first to investigate the associations between sleep and fatigue in children with FMF, to the best of our knowledge. Our results suggest that fatigue was associated with poor sleep quality and less sleep duration. Children who were sleeping less than seven hours reported considerably worse fatigue (more than 30%). Thus, evaluating sleep alongside fatigue can help to understand the problems of the patients better.

Pediatric and adult rheumatic diseases are known to lead to an inactive lifestyle.[32-34] Thus, measuring physical activity has become an important part of patient assessment due to the general and disease-related health benefits of physical activity in rheumatic conditions.[35] However, no study has investigated physical activity in children with FMF to date. Results of the previous studies conducted in adult patients with FMF revealed that patients were prone to engage in less physical activity, even during attack-free periods.[36] According to the results of present study, performing less physical activity was significantly associated with higher fatigue levels, and children who had an inactive lifestyle reported higher fatigue. Nonetheless, the direction of this relationship (if the children were inactive due to fatigue or vice versa) is still unknown. As the present study was performed during and recently following Covid-19 period, the pandemic itself and related previous curfews may be other possible factors for limiting physical activity and fatigue eventually.[37] Cardiorespiratory and/or musculoskeletal systems which are essential for fatigue were also reported to be affected by Covid-19 pandemic.[38] Although, none of the participants had Covid-19 during their participation in the study, a previous Covid-19 history was not an exclusion criteria. This could have played a role in self-reported fatigue levels by some children in the present study.

This study has some potential limitations. Only adolescents (aged 13-18 years) were included in the present study. Although the questions are identical in forms for other age groups, fatigue perception in younger children with FMF might be different from adolescents. Thus, our results should be confirmed with other age groups. Drug compliance was inquired verbally in the present study, however, a structured drug compliance assessment might help in interpreting the results more clearly. Besides, the disease activity status was not evaluated thoroughly, and adolescents were considered in attack-free period according to research pediatricians' opinions. Moreover, the number of participants in 'no *M694V* mutation' and 'no colchicine' subgroups were too low to reach a strict conclusion. Future studies with larger sample sizes may be helpful for better understanding the true effect of these variables on fatigue. Evaluating physical activity and sleep-related parameters objectively would also have enhanced our results. Lastly, discriminant validity of PedsQL could not be investigated due to lack of a healthy control group.

PedsQL-MFS is a valid and reliable tool for evaluating fatigue in children with FMF. Being female, recent attacks, physical inactivity, and sleeping less than seven hours seem to be associated with higher fatigue.

## Ethical approval

The study was approved by the İzmir Katip Çelebi University, Ethical Committee on 24.06.2021, with number: 0308. All the patients signed informed written consent for publication/participation before the study.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: DCS, DB; data collection: SM, BKD, ÖAG, İİ, İA,

CHK, SK, EP; analysis and interpretation of results: DCS, BKD, ÖAG, DB; draft manuscript preparation: DCS, DB. All authors reviewed the results and approved the final version of the manuscript.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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**Original Article**

# The effects of glucocorticoid plus intravenous immunoglobulin (IVIG) vs IVIG alone on platelet activation in children with Kawasaki disease

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## ABSTRACT

**Background.** Even though intravenous immunoglobulin (IVIG) is a current treatment for Kawasaki disease (KD), 10–20% of patients require additional therapy. This study seeks to investigate the therapeutic effects of glucocorticoids plus IVIG on KD and to ascertain the subsequent effect on platelet activation during the acute phase.

**Methods.** A total of 32 children with KD were randomly classified into two groups: the experimental group (16 cases) and the control group (16 cases). The control group was exposed to IVIG (2 g/kg), whereas children in the experimental group were treated with IVIG (2 g/kg) + glucocorticoid. Peripheral venous blood samples were obtained from all participants before treatment as well as three days post-treatment to test platelet activation levels with procaspase activating compound-1 (PAC-1) antibody, Toll-like receptor 4 (TLR4), interleukin-6 (IL-6), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), procalcitonin (PCT), and C-reactive protein (CRP). Fever duration post-treatment was documented for both groups. Additionally, the coronary arteries in both groups were evaluated during three months of treatment.

**Results.** After treatment, the experimental group had remarkably lower levels of TNF-$\alpha$, CRP, PCT, IL-6, PAC-1, and TLR4 relative to the control group. The fever persistence rate was considerably elevated in the control group compared to the experimental group (log-rank, P=0.024). In addition, the z-score of coronary artery size dropped after IVIG + glucocorticoids treatment compared to the control group, although this difference was not significant.

**Conclusions.** The IVIG + glucocorticoids can quickly mitigate the inflammatory response and platelet activation. Moreover, it can also improve clinical symptoms in children with KD.

**Key words:** Kawasaki disease, corticosteroid, intravenous immunoglobulin, platelet activation, coronary artery lesions.

Kawasaki disease (KD) is an acute self-limiting inflammatory disorder related to systemic vasculitis.[1] Coronary vessel wall inflammation in KD can cause coronary artery abnormalities (CAAs).[1] Moreover, coronary vascular endothelium damage can potentially activate platelets, thereby initiating a cascade of further vascular damage.[2-4] Literature studies depict that platelet activation and systemic inflammatory changes during the acute phase of KD support the release of procaspase activating compound-1(PAC-1) and Toll-like receptor 4 (TLR4), thereby accelerating disease progression.[5,6] Hence it provides a theoretical basis for the treatment of KD with an antagonist that modulates platelet-activating factors and suppresses inflammatory response.

Exposure to intravenous immunoglobulin (IVIG) reduces the incidence of coronary aneurysms from 25% to only nearly 9%.[7] Even with timely IVIG treatment, around 4% of affected children

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progress to coronary anomalies, and 1% can even develop giant aneurysms.[8] A recrudescent or persistent fever following infusion with IVIG is one of the most powerful risk factors for a coronary aneurysm - IVIG resistance.[9] About 15-20% of patients develop IVIG resistance at the end of IVIG therapy and are considered more susceptible to developing CAAs. IVIG resistance rates have consistently increased from about 7% in 2003 to ~23% in 2014, with a concomitant elevation in aneurysms of the coronary artery.[10] Several treatment strategies have been tested to further decrease the risk of coronary artery aneurysms.[11-13] The addition of steroids to conventional treatment is one option; however, the effect remains controversial. A potential positive effect of glucocorticoids in the acute phase of KD has been suggested in recent studies.[13-16] This study aimed to demonstrate the impact of glucocorticoids plus IVIG in KD treatment and characterize its potential mechanisms in the acute phase of the disease.

## Material and Methods

### *Study design*

*Inclusion criteria:* 1) All patients meeting the KD diagnostic criteria defined by the American Heart Association (AHA) in 2017[1] : a) fever ≥5 days, b) conjunctival hyperemia in both eyes, c) oral changes, d) erythema multiforme and rashes, e) hand and foot sclerosis or peeling, f) acute non-suppurative lymphadenitis in the neck. KD was diagnosed in the presence of at least 5 of the six principal symptoms. 2) Patients without KD-specific treatment prior to admission. 3) Individuals with family members willing and able to cooperate for study completion. 4) Individuals who granted written informed consent via forms.

*Exclusion criteria:* 1) Individuals with concomitant severe congenital or organic diseases of the liver, heart, or kidney. 2) Individuals with a history of KD. 3) Individuals presenting symptoms of coronary artery abnormalities prior to admission. 4) Individuals who had received intravenous immunoglobulin or steroids prior to admission. 5) Individuals who took related drugs like immunosuppressants, which might influence the study.

### *Randomization and blinding*

The randomization technique employed in this study involved a sealed envelope system. Informed consent was obtained from the parents of children who met the inclusion criteria, and treatments were randomly assigned to sealed envelopes. Following this, a physician opened a random envelope and selected the assigned treatment regimen. Patients and clinicians were not blinded to the assignment.

### *Study population*

A total of 32 children diagnosed with KD were enrolled in the study at the Provincial Children's Hospital Affiliated with Anhui Medical University, from January 1, 2020, to December 31, 2021, with 16 cases allocated to each group. Patient baseline characteristics are shown in Table I. The Institutional Ethics Committee of the Provincial Children's Hospital, Anhui Medical University approved the study. Moreover, informed, signed consent was obtained from all involved.

Treatment groups: Individuals in the control group were administered IVIG and aspirin. The exact treatment regimen included immunoglobulin 2g/kg, intravenous drip, and single-dose application. Simultaneously, aspirin was orally administered at 30-50 mg/kg/day, gradually declining to 3-5 mg/kg/day once the fever subsided for at least three consecutive days. These conditions were maintained for two months. On the other hand, individuals in the experimental group were exposed to glucocorticoids in addition to IVIG and aspirin, as above. The glucocorticoids scheme was intravenous methylprednisolone at a dose of 2 mg/kg twice daily for three days. After a 3-day fever-free period, the oral prednisolone dose

**Table I.** Clinical and laboratory characteristics of experimental and control participants

| Characteristics | Experimental group (n=16) | Control group (n=16) | P-value |
|---|---|---|---|
| Age (months) | 32.0 (20.3-45.0) | 27.0 (13.0-36.0) | 0.509 |
| Male/female | 9/7 | 11/5 | 0.716 |
| Weight (kg) | 12.0 (9.6-15.6) | 12.0 (9.5-14.0) | 0.721 |
| Days of illness at treatment (day) | 5.1±1.9 | 5.1±1.9 | 0.927 |
| Hemoglobin(g/dl) | 10.52±1.27 | 11.30±1..27 | 0.092 |
| White-cell count ($\times10^3/\mu L$) | 13.0 (10.3-15.5) | 15.2 (11.3-17.2) | 0.122 |
| Platelet count ($\times10^4/\mu L$) | 312.1±127.3 | 375.7±145.3 | 0.198 |
| D-dimer(mg/L) | 1.5 (1.1-2.1) | 1.1 (1.0-2.8) | 0.228 |
| CD64 (%) | 5.8 (4.8-8.2) | 6.1 (5.5-7.9) | 0.427 |

was changed to 2 mg/kg. Prednisolone was also gradually reduced in 5-day steps over 15 days from 2 mg/kg/day to 1 mg/kg/day to 0.5 mg/kg/day. The total course of steroids was 18 days.

The discharge summary of the patients included medication instructions, the need for regular follow-ups, and regular notifications at each point of the assessment. Furthermore, the patients were consistently reminded to undergo relevant examinations at every assessment. At the end of each follow-up, the physician also ensured that the patient was informed about the schedule and contents for the next visit.

### *Sample collection*

Clinical data, including age, height, weight, gender, and time of body temperature drop after treatment, were obtained. Clinical inflammatory markers procalcitonin (PCT), C-reactive protein (CRP), tumor necrosis factor-$\alpha$ (TNF-$\alpha$), and interleukin-6 (IL-6), were obtained from patients before treatment as well as three days post-treatment. The assessments of coronary arteries were carried out by measuring the luminal diameter of the main right and left coronary arteries, the left anterior descending artery, and the left circumflex artery before treatment, 2-, 4-, and 12- weeks after treatment.

### *Platelet activation detection by flow cytometry*

Blood samples were obtained from the patients before IVIG and three days after IVIG. A 2 ml blood draw was acquired from patients and controls using a 5 ml needle and immediately transferred to plastic tubes containing EDTA. This was done to avoid manual activation of the platelets during sample collection. Additionally, during transport from the clinic to the laboratory, samples were kept at room temperature to eliminate any effect of temperature fluctuations on platelet activation. These samples were then centrifuged for 10 min. at 800 X g to obtain platelet-rich plasma (PRP). This step was followed by immediate freezing at -80°C until further analysis.

All samples were immobilized with 1% paraformaldehyde and analyzed on a FACSCanto II flow cytometer (Becton Dickinson, USA). Reagents like mouse anti-human CD61 antibody conjugated to BV510, mouse anti-human TLR4 antibody conjugated to phycoerythrin (PE), and mouse anti-human PAC-1 antibody conjugated to APC were purchased from B.D. Pharmingen (USA). As for the controls, immunoglobulins from the same mouse species were used (B.D. Pharmingen, USA) for flow cytometry. Furthermore, the CD61 marker served as an activation-independent marker of platelets. The percentage of platelets expressing TLR4 or PAC-1 was considered the fraction exhibiting specific binding (TLR4 or PAC-1 positive) minus non-specific binding (the percentage with IgG-PE conjugate) of the 10,000 platelets analyzed. Moreover, the TLR4 or PAC-1 expression assays were performed in duplicates for each blood sample, and the means were recorded.

### *Endpoints*

The primary endpoint: levels of PAC-1 and TLR4 before treatment as well as three days after treatment.

Secondary endpoints: 1) z-scores of coronary arteries, 2) duration of fever post-treatment, 3) levels of TNF-$\alpha$, IL-6, CRP, and PCT at three days post-treatment, 4) incidence of side effects during the treatment.

### *Outcomes*

The primary outcome: changes in platelet activation levels at three days following treatment.

Secondary outcomes: 1) duration of fever (hours): from completion of initial IVIG infusion to afebrile condition, 2) changes of z-scores of coronary artery throughout the study period, 3) changes in TNF-$\alpha$, IL-6, CRP, and PCT levels at three days post-treatment, 4) Frequency of all side effects during the treatment.

### *Statistical analysis*

IBM SPSS version 22.0 (USA) aided the statistical analyses. For continuous variables, the data were depicted as the mean plus or minus standard deviations or as the median and interquartile range for normal and non-parametric data, respectively. On the other hand, categorical variables were expressed as frequencies and proportions. Data were compared by means of the Student's t-test and repetitive measure analysis of variance for normally distributed continuous variables. In contrast, Fisher's exact test was employed for categorical variables.

Furthermore, non-parametric continuous variables were assessed by means of the Mann-Whitney U test or the Wilcoxon signed-rank test. Following this, sequential persistence curves were computed by applying the Kaplan-Meier method and were subsequently compared using the log-rank test. All tests were two-tailed, and a P-value of <0.05 was considered significant.

## Results

### *Changes in inflammatory factors*

Alterations in inflammatory factors both prior to treatment and post-treatment between the two groups are depicted in Table II, which demonstrates that TNF-$\alpha$, CRP, IL-6, and PCT were remarkably elevated before treatment in the two groups. However, the difference was not significant (P>0.05). Moreover, the above-mentioned markers declined at three days post-treatment, with a statistically significant difference (P<0.05). The experimental group had remarkably lower levels of TNF-$\alpha$, CRP, PCT, and IL-6 relative to the control group (p<0.05).

**Table II.** Analysis of changes in inflammatory factors before and after treatment according to treatment groups

| Variables | | Experimental group (n=16) | Control group (n=16) | P |
|---|---|---|---|---|
| CRP (mg/L) | Before treatment | 71.49 (42.1,119.38) | 64.15 (51.91,85.38) | 0.534 |
| | 3d after treatment | 4.83 (0.5,7.84) | 7.64 (4.28,11.98) | 0.047 |
| | P | <0.001 | <0.001 | |
| PCT (ng/ml) | Before treatment | 0.79 (0.28,4.63) | 0.25 (0.16,1.45) | 0.250 |
| | 3d after treatment | 0.05 (0.04,0.09) | 0.16 (0.06,0.38) | 0.021 |
| | P | 0.001 | 0.026 | |
| IL-6 (ng/L) | Before treatment | 158.38 (39.04,544.08) | 86.45 (41.80,263.35) | 0.059 |
| | 3d after treatment | 2.00 (1.50,3.04) | 3.36 (2.24,8.65) | 0.023 |
| | P | <0.001 | <0.001 | |
| TNF-$\alpha$ (ng/L) | Before treatment | 18.75 (12.70,29.13) | 17.05 (10.73,22.31) | 0.402 |
| | 3d after treatment | 6.20 (4.00,11.53) | 12.15 (9.28,16.50) | 0.020 |
| | P | 0.001 | 0.031 | |

### *Expression levels of PAC-1 and TLR4*

Expression levels of platelet activation in the two groups are depicted in Fig. 1. The PAC-1 and TLR4 antibody targeting GPIIb/IIIa levels in both groups were remarkably elevated prior to treatment, and the difference between them was not significant (P>0.05). The two indicators declined post-treatment, with a statistically significant drop from before treatment (P<0.05). Moreover, the levels of TLR4 and PAC-1 in the experimental group were significantly lower than those in the control group at three days post-treatment (P<0.05).

### *Time-dependent change of the fever in patients with and without glucocorticoid therapy*

Duration of fever after treatment between the two groups was determined. The Kaplan-Meier curves for the rate of cumulative persistence of fever are illustrated in Fig. 2. The 5-, 10-, 20-, 30-, 60-, and 70-hour rates of cumulative persistence of the fever were 31.3%, 6.3%, 6.3%, 0%, 0%, and 0%, respectively in the experimental group. On the contrary, the observed percentages were 50%, 25%, 12.5%, 6.3%, 6.3%, and 0%, respectively, in the control group. Furthermore, the rate of fever persistence was significantly elevated in the control group relative to the experimental group (log-rank, P=0.024). The median fever duration regression in the experimental group was 3.0 hours, whereas it was 4.5 hours for the control group.

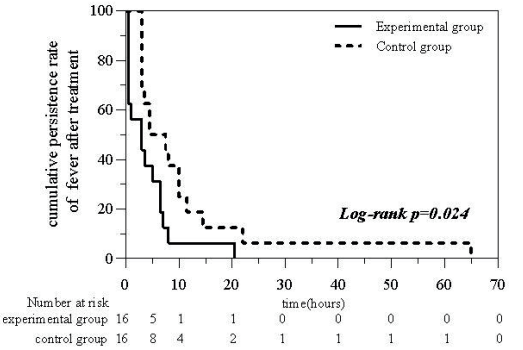


**Fig. 2.** Kaplan-Meier analyses for the cumulative persistence rate of fever after treatment.

### *The change in coronary artery z-scores over time*

The change in coronary artery z-score prior to treatment relative to post-treatment between the two groups is illustrated in Fig. 3. The z-score declined sharply following IVIG + glucocorticoid therapy relative to the control group. However, the difference was not significant (P>0.05).

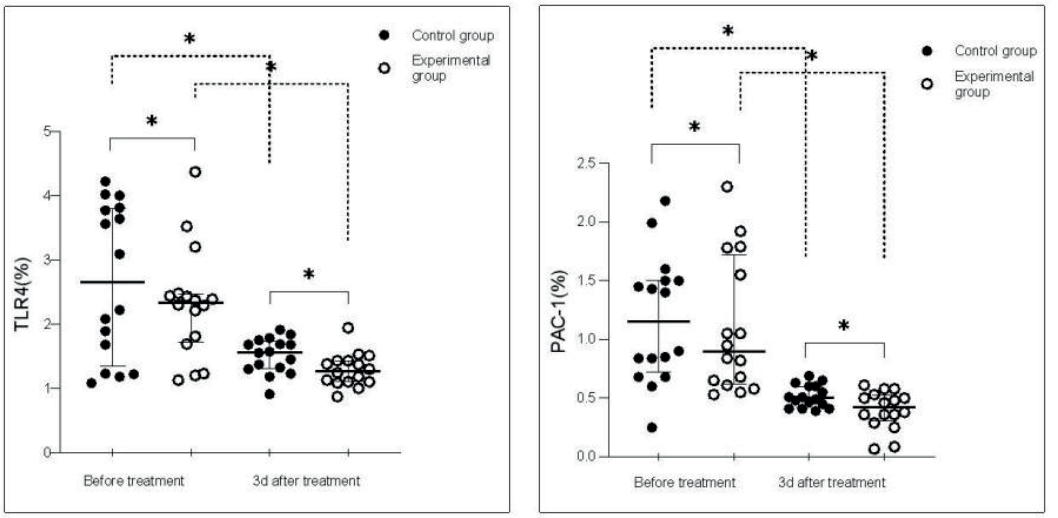


**Fig. 1.** Scatterplots of TLR4 (a), and PAC-1(b). Values depict the median and interquartile range of individuals in the two groups. * P<0.05

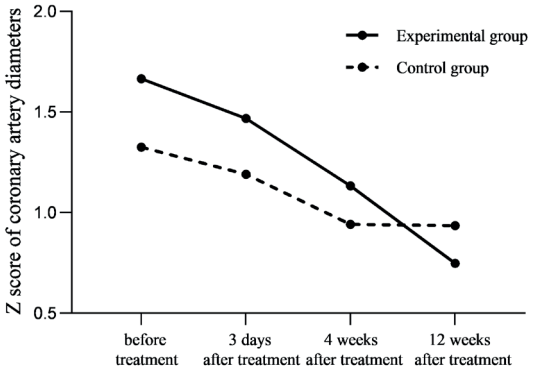


**Fig. 3.** Mean z-score levels of coronary artery diameters over time.

During the treatment, no significant side effects or adverse reactions to the drug were observed in any of the study participants.

## Discussion

Coronary artery lesions (CALs) are among the most significant KD-related complications as well as a major determinant of long-term prognosis.[14] CALs can cause aneurysms of the coronary artery, myocardial ischemia, occlusions, myocardial infarction, or even death.[17] The vascular injury mechanism in children with KD is hypothesized to be linked to the activation of the monocyte/macrophage system *in vivo*. This releases several inflammatory mediators and factors, thereby causing vascular endothelial injury. Additionally, several studies establish that IL-2, 4, 6, 8, and 17 are positively correlated with the inflammatory response in the acute phase of KD. IL-6 even contributes to CAAs formation and vascular endothelial damage.[18-20] In this experiment, IL-6, TNF-$\alpha$, PCT, and CRP levels were remarkably elevated in children with KD prior to treatment, which is a high-level inflammatory response in the acute phase of KD.

Corticosteroids are a relatively safe and affordable option for most individuals as adjunctive therapy for the primary treatment of KD. In the study by Burns[21], the author demonstrated an advantage in aneurysm size reduction among individuals treated with pulse methylprednisolone. This finding ensued global interest regarding steroid function in the treatment of acute KD.[21] A recent study showed that compared to IVIG alone, glucocorticoid combined with IVIG rapidly reduces TNF-$\alpha$, IL-6, and CRP levels in individuals with KD.[13] Another prospective study showed that the addition of prednisolone to IVIG treatment reduced the inflammatory response in patients with KD.[15] In addition, this treatment regimen resulted in faster fever resolution than IVIG alone. This study confirmed similar results. With oral administration of aspirin, individuals exposed to glucocorticoids plus IVIG showed remarkably lower levels of inflammatory factors (such as TNF-$\alpha$, IL-6, PCT, and CRP), compared to those who received conventional immunoglobulin therapy ($p < 0.05$). The rate of cumulative persistence of fever was also lower in the glucocorticoids plus IVIG category than in IVIG alone (log-rank, $p=0.024$). These findings strongly depict that the treatment regimen coupled with corticosteroids is more conducive to alleviating the inflammatory state and improving clinical symptoms in individuals with KD.

Prospective research revealed that anomalies in the coronary artery were significantly reduced in the IVIG plus prednisolone category than with IVIG alone. The authors suggest that the duration of steroid treatment for KD might be more relevant than the maximum concentration.[22] However, a subsequent study was unable to show a positive effect of steroid treatment on reducing CAA.[23] The reasons for the considerable variation between the two studies are (1) individuals with incomplete KD and (2) individuals with z-scores ≥2.5 for the initial coronary artery. In this study, the z-scores of coronary artery size post-treatment with IVIG + glucocorticoids were smaller relative to IVIG alone. However, these variations were not significant, probably due to the small sample size. Thus, further studies are needed to verify this finding. Although the difference was not

statistically significant, it indicates ways to mitigate CALs.

The PAC-1 monoclonal antibody identifies a conformational alteration in the GPIIb/IIIa complex on activated platelets.[24] This conformational change is a key step in platelet activation and leads to platelet aggregation by various pathways.[25] The vascular endothelial growth factor (VEGF) is released from platelets during whole blood clotting. It serves a crucial function in regulating angiogenesis.[19] Studies show that VEGF is highly expressed in the serum of children with KD during an early stage of vascular inflammation and is involved in the formation of CALs.[19,26] Platelet activation causes a conformational alteration in the GpIIb/IIIa complex. This, in turn, exposes almost 80,000 fibrinogen binding sites on the surface of the platelet.[27] Fibrinogen binding to these receptors might be a prerequisite for VEGF release.[28] Moreover, the activation of TLR4 leads to exacerbated platelet responses in KD patients, potentially contributing to atherogenesis through the delivery of proinflammatory factors to leukocytes and endothelial cells.[29,30] Furthermore, TLR4 induces nuclear factor-κB (NF-κB)-dependent cytokine production through the myeloid differentiation primary-response gene 88 (MyD88) pathway.[31,32] TLR4 signaling through NF-κB also contributes to the pathology of vascular injury in individuals with KD.[33]

Moreover, recent studies have shown that platelets are highly activated in KD patients, which is probably one of the most crucial pathophysiological steps in the disease.[1,34] Therefore, it is vital to assess whether there is any change in the status of platelets in KD patients post-treatment with glucocorticoid. Ueno and his colleagues[35] observed platelet activation levels as significantly elevated in individuals with KD and CAA versus individuals without CAAs. Additionally, platelet activation levels were considerably lower in individuals with KD exposed to both IVIG and oral prednisolone in comparison with individuals who had received IVIG alone.[35] The present study confirmed the presence of platelet activation in individuals with KD. It also found remarkably lower levels of PAC-1 and TLR4 in the glucocorticoid plus IVIG group relative to the control group ($p < 0.05$). These findings establish that reducing platelet activation is a function of glucocorticoids in KD. Furthermore, Yahata et al.[36] found that high levels of platelet activation were still present in children recovering from KD for 2-3 months, which may explain the better effect of long-term steroids than short-term steroids in children with KD.

Limited research has been conducted on the impact of glucocorticoids in reducing platelet activation. However, it is speculated that glucocorticoids function by inhibiting the pathway that stimulates platelet activation through the inflammatory response.[37] Glucocorticoid downregulates VEGF expression in the serum by inhibiting platelet activation, thereby suppressing vascular injuries in KD. Additionally, glucocorticoids also block the TLR4 signaling pathway by inhibiting platelet activation, which in turn, inhibits phosphorylation of NF-κB, thereby suppressing its activation. Furthermore, glucocorticoids interfere with the inflammatory response in KD by inhibiting inflammatory molecule production through the downregulation of NF-κB levels.[38]

Although this study demonstrates the use of glucocorticoids in KD treatment, the sample size was relatively small. Hence, there was no strict long-term follow-up of results, which limits the obtained insights. Future studies will need to expand the sample size and increase the post-treatment follow-up duration to accurately ascertain the long-term impact of glucocorticoids on the prognosis of individuals with heart damage.

In summary, glucocorticoids plus IVIG therapy may inhibit the inflammatory response and platelet activation and aid in vascular remodeling. Additional prospective studies

are needed to further investigate the efficacy of glucocorticoids plus IVIG therapy in the acute phase of KD.

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**Ethical approval**

The study was licensed by the Institutional Ethics Committee of the Provincial Children's Hospital and the Anhui Medical University. Additionally, signed, informed consent was obtained from all involved (Approval no: EYLL-2019-023).

**Author contribution**

The authors confirm their contribution to the paper as follows: study conception and design: QQW, LYZ, SZ; data collection: QQW; analysis and interpretation of results: QQW, SZ; draft manuscript preparation: QQW. All authors reviewed the results and approved the final version of the manuscript.

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**Conflict of interest**

The authors declare that there is no conflict of interest.

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**Original Article**

# Evaluation of common NLRP3 Q703K variant in pediatric patients with autoinflammatory disease: CAPS and PFAPA

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## ABSTRACT

**Background.** Gain-of-function mutations of the NLR family pyrin domain containing 3 (*NLRP3*) gene have been implicated in autoinflammatory diseases. The *NLRP3* Q703K variant is a common variant associated with Cryopyrin-associated periodic syndromes (CAPS) and periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome. However, the genotype-phenotype correlation between *NLRP3* Q703K variant, CAPS and PFAPA is unclear. In this study, we aimed to investigate the frequency of the *NLRP3* Q703K variant in patients with and without autoinflammatory disease and characterize the phenotype in only Q703K variant positive patients.

**Methods.** A retrospective analysis of 639 patients with autoinflammatory symptoms was conducted. Patients underwent next-generation sequencing (NGS) panel analysis of 16 genes, including *NLRP3*. For the 68 patients carrying the only Q703K variant, their clinical and demographic information was evaluated. Genetic data from 1461 patients without autoinflammatory symptoms were used as the control group.

**Results.** Of our 639 autoinflammatory symptomatic patients, the Q703K mutation was detected in 68 (5.3% allele frequency). Heterozygous mutations were detected in 141 patients without autoinflammatory symptoms (4.8% allele frequency, p=0.4887). Of the patients with variant in Q703K, 10 patients were diagnosed with CAPS , 7 patients were diagnosed with PFAPA and the remaining 39 were diagnosed with undefined systemic autoinflammatory disease (uSAID)

**Conclusions.** The Q703K variant, which is seen with similar frequency in the control and autoinflammatory groups, is also of higher prevalence in patients with mild CAPS symptoms and PFAPA syndrome. This variant, together with other undetected genetic variants or epigenetic modifications, may be responsible for the corresponding phenotype. As such, it is essential for clinicians to evaluate their patients using both genetic and clinical evaluations.

**Key words:** cryopyrin-associated periodic syndromes; periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis; NLRP3 gene; Q703K variant.

Systemic autoinflammatory diseases (SAIDs) are clinically defined by the recurrence of multisystemic inflammatory attacks without infection or autoantibody formation.[1] The term undefined systemic autoinflammatory diseases (uSAIDs) is used with increasing frequency in patients with a deficient phenotype, although there is no defined diagnostic criterion.[2,3]

Cryopyrin-associated periodic syndromes (CAPS) includes 3 clinically overlapping entities, namely, from familial cold-induced autoinflammatory syndrome 1 (FCAS, OMIM #120100), Muckle-Wells syndrome (MWS, OMIM #191900) and chronic infantile neurological, cutaneous, and articular syndrome (CINCA, OMIM #607115) with a broad clinical spectrum of severity. Patients

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with FCAS have a mildest phenotype.[1,4-6] CAPS are caused by gain-of-function mutations in the NLR family pyrin domain containing 3 (*NLRP3)* gene, which is a key component of the interleukin-1 (IL-1) inflammasome.[7] Recurrent episodes of fever, myalgia, abdominal pain, arthralgia, cutaneous inflammation, ocular and central nervous system involvement are common symptoms in cases with pathogenic mutations.[8-10] One low-penetrance variant in particular, Q703K (rs35829419, c 2107C>A, p.Gln703Lys) (also known in the literature as Q705K), is seen with a similar frequency in both pathogenic patients and healthy individuals, calling its functional significance into question.[11-13] Due to Q703K noted as high frequency in the general population (5-11%), some studies have accepted it as a clinically unremarkable polymorphism.[14,15]

Other studies, however, report patients carrying the Q703K variant as pathogenic, given that they have notably high levels of IL-1β.[13,16] This hypothesis was supported by the detection of the Q703K mutation in 7 patients with CAPS-like symptoms.[17]

Periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis (PFAPA) syndrome was first described in 1987 and is characterized by fever, oral aphthosis pharyngitis and cervical lymphadenopathy. Though its symptoms are well defined, its etiology is not as clearly understood.[18,19] When screening for PFAPA, detection of positive family history in pedigree suggests a similar genetic background.[20] *NLRP3* variants have been detected in approximately 20% of patients with PFAPA syndrome, and IL-1β monocyte production has been shown to be irregular, suggesting that inflammatory genes may be involved in this autoinflammatory syndrome.[7] The Q703K variant appears common in healthy populations and its pathogenic significance is unclear. It has been found to be associated with PFAPA and CAPS syndrome.[21]

In this study, we aimed to investigate the frequency of the Q703K variant in patients with autoinflammatory disease and in the control group without autoinflammatory symptoms. Furthermore, we aimed to characterize the phenotype in only Q703K variant positive patients.

## Material and Methods

### *Patient selection*

A retrospective analysis was carried out by surveying pediatric rheumatology and pediatric genetic patients' data between the years of 2016 and 2020 at the University of Health Sciences Ümraniye Education and Research Hospital, İstanbul, Türkiye. This study was approved by the local Ethics Committee of the same hospital (Approval Number: B.10.1.TKH.4.34.H.GP.0.01/07/26.01.2023). Consent was obtained from the patients' legal guardians according to the Declaration of Helsinki. The 639 Turkish origin patients with autoinflammatory symptoms (including but not limited to recurrent and periodic fever, mouth ulcer, rash, abdominal pain, and arthralgia) and no history of familial mediterranean fever (FMF) who applied to the pediatric rheumatology clinic between 2016 and 2020 underwent a next-generation sequencing (NGS) autoinflammatory panel containing 16 genes. Of these patients, 68 had heterozygous NLRP3 Q703K variant. After a 1-year follow-up, 56 with complete data were evaluated. The preliminary diagnosis was made on The Eurofever clinical diagnostic/classification criteria- Feredici score and Modified Marshall's diagnostic criteria for SAIDs and PFAPA syndrome.[22,23] Patients who were excluded for any of monogenic SAID or PFAPA syndrome were classified as uSAIDs. The final diagnosis of the patients was made using the New Eurofever/PRINTO classification and Modified Marshall's diagnostic criteria, with the results of the autoinflammatory panel and clinical symptom and attack followed-up for at least one year.[23,24] Clinical, demographic and laboratory information were obtained from the information recorded by pediatric rheumatology for all patients whose genetic

panel testing was only positive for the Q703K variant. None of these patients had heterozygous or homozygous pathogenic/likely pathogenic FMF variants. The cutoff values determined by our laboratory were 0.5 mg/dl for C-reactive protein (CRP) and 20 mm/h for the erythrocyte sedimentation rate (ESR).

Data from 1461 patients who did not have any history of autoinflammatory symptoms but who had clinical exome analysis for other reasons (i.e., skeletal dysplasia, dysmorphic features, development delay, etc.) and a high average age with adult patients were included as the control group.

### *Genetic testing and analysis*

Genomic DNA was extracted from EDTA-anticoagulated peripheral blood using a semi-automated robot, as recommended by the manufacturer (Qiagen). The concentration and quality-control (260/280 nm and 260/230 nm absorbance ratios) of the DNA samples were determined by spectrophotometrically (Nanodrop 2000, Thermo Scientific, USA) and fluorometrically (Qubit v3.0, Thermo Fisher, USA). The library preparation for NGS was performed using Fever &Autoinflammatory Diseases Kit by Sophia-Genetics, a custom panel using a capture-based method. panel containing 16 genes (*MEFV, MVK, NLRP3, NLRP12, TNFRSF1A, TNFRSF11A, LPIN2, PSTPIP1, IL1RN, CECR1, ELANE, CARD14, IL10RA, IL10RB, NOD2, and PSMB*8). NextSeq-500-(Illumina) was used as the sequencing platform. In the control group, the library preparation for NGS was performed using a capture-based Clinical Exome Solution Kit by Sophia Genetics, that included 4900 genes. Data quality control, alignment, variant calling and variant annotations were performed using the Sophia DDM analysis tool (version5.2). NCBI Build37 (hg19) version of the human genome was used as a reference. As a primary variant filtering strategy, variants located within the ± 10 base pair boundary of targeted exons with minimum read depth 50× were selected. Any variants outside these regions, variants in homopolymer regions and exonic variants with a variant fraction of less than 20% were considered false positives and were not analyzed. All variants were manually inspected by using IGV visualization tool. We confirmed NLRP3 Q703K mutation in 10 samples by Sanger sequencing and omitted the remaining ones that had well coverage and mappability on manual IGV inspection.

### *Statistical analysis*

Categorical data are presented as numbers and percentages. Statistical package for the social sciences (SPSS) (version 230, SPSS-Inc., Chicago, IL, USA) was used for statistical analysis. Categorical data are presented as numbers and percentages. Numerical data with asymmetrical distribution are presented as the median and interquartile range (IQR). Fisher's exact test was used to compare quantitative data. A p-value less than 0.05 was considered statistically significant.

## Results

Within the pool of 639 recruited pediatric rheumatology patients, 68 carried the heterozygous Q703K mutation in the *NLRP3* gene. The overall allelic frequency was 5.3%. In order to evaluate the allele frequency of the Q703K mutation in the general population, the data and clinical files of 1461 patients who were evaluated at the genetic diagnosis center for other reasons (multiple congenital anomalies, severe growth retardation, etc.) were examined. Heterozygous mutations were detected in 141 patients without autoinflammatory symptoms. The allele frequency in individuals without autoinflammatory symptom complaints was found to be 4.8% (p=0.4882).

Of the 68 patients with Q703K, 56 (82.3%) had complete clinical data during molecular analysis and at follow-up. Within these 56 patients, they were further classified into CAPS mild phenotype (n=10), PFAPA (n=7) and

**Table I.** Clinical data of patients with Q703K mutation.

| P N | Fever | Abdominal pain | Skin rash | Artralgia /arthritis | Conjunctivitis | Family history | Pharangitis | Tonsillitis | Aphthous stomatitis | Adenitis | Headache | Myalgia | Hearing loss | Elevated acute phase reactans | Preliminary diagnosis | Final diagnosis | Treatment | Genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | + | - | + | -/- | - | - | - | - | - | - | - | - | - | - | CAPS | CAPS | C, IL-1Ra | Q703K/WT |
| 2 | + | - | + | +/- | - | - | - | - | - | - | - | - | - | + | CAPS | CAPS | C, IL-1Ra | Q703K/WT |
| 3 | + | - | + | -/- | - | - | - | - | - | - | - | - | - | - | CAPS | CAPS | C | Q703K/WT |
| 4 | + | - | + | +/- | - | - | - | - | - | - | - | - | - | + | CAPS | CAPS | C | Q703K/WT |
| 5 | + | + | - | -/- | + | - | - | - | - | - | - | - | - | - | uSAID | CAPS | C | Q703K/WT |
| 6 | + | - | + | -/- | - | - | - | - | - | - | - | + | - | + | CAPS | CAPS | C | Q703K/WT |
| 7 | - | - | + | +/- | + | + | - | - | - | - | - | - | - | + | CAPS | CAPS | C | Q703K/WT |
| 8 | - | + | - | +/- | + | + | - | - | - | - | + | - | - | + | uSAID | CAPS | C | Q703K/WT |
| 9 | + | + | + | -/- | - | - | - | - | - | - | - | + | - | + | uSAID | CAPS | C | Q703K/WT |
| 10 | + | - | - | -/- | + | NA | - | + | + | + | + | - | - | + | CAPS | CAPS | C | Q703K/WT |
| 11 | + | - | - | +/- | - | - | + | + | - | - | + | - | - | NA | uSAID | PFAPA | C | Q703K/WT |
| 12 | + | - | - | -/- | - | - | - | + | + | - | - | - | - | + | uSAID | PFAPA | C | Q703K/WT |
| 13 | + | + | - | -/- | - | + | + | + | - | - | - | - | - | + | PFAPA | PFAPA | C | Q703K/WT |
| 14 | + | + | - | -/- | - | - | - | - | + | - | - | - | - | + | uSAID | PFAPA | P | Q703K/WT |
| 15 | + | - | - | -/- | - | - | - | + | + | + | - | - | - | - | PFAPA | PFAPA | P | Q703K/WT |
| 16 | + | - | - | -/- | - | - | - | + | + | - | - | - | - | - | PFAPA | PFAPA | P | Q703K/WT |
| 17 | + | - | + | -/- | - | - | + | - | - | - | - | - | - | + | PFAPA | PFAPA | C | Q703K/WT |
| 18 | + | + | + | -/- | - | - | - | - | - | - | - | - | - | + | uSAID | uSAID | - | Q703K/WT |
| 19 | + | - | + | -/- | - | + | - | - | - | - | - | - | - | + | CAPS | uSAID | C | Q703K/WT |
| 20 | + | + | - | -/- | - | - | - | - | + | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 21 | + | + | - | -/- | + | - | - | - | - | - | + | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 22 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 23 | + | - | + | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 24 | + | - | - | +/- | - | - | - | + | - | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |
| 25 | + | + | - | -/- | - | + | - | - | - | - | - | - | - | - | uSAID | uSAID | - | Q703K/WT |
| 26 | + | - | - | -/- | - | - | - | - | - | - | - | - | - | + | HIDS | uSAID | C | Q703K/WT |
| 27 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |
| 28 | + | - | - | -/- | - | - | - | + | - | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |
| 29 | - | + | - | -/- | - | - | - | - | - | - | - | - | - | - | TRAPS | uSAID | C | Q703K/WT |
| 30 | + | + | - | +/- | - | - | - | - | - | - | - | - | - | + | TRAPS | uSAID | C | Q703K/WT |
| 31 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 32 | + | - | - | -/- | - | - | - | + | - | - | - | - | - | NA | uSAID | uSAID | C | Q703K/WT |
| 33 | + | + | - | -/- | - | - | - | + | + | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |
| 34 | + | - | - | -/- | - | - | - | - | - | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |

CAPS:Cryopyrin-associated periodic syndromes, HIDS: Hyperimmunoglobulin D Syndrome, PFAPA: periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis, IVIG: intravenous immunoglobulin, NA: Not available, PN: Patient number, WT: Wild type, uSAID: Undefined systemic autoinflammatory disease, C: colchicine, P: Predinisolone, TRAPS: Tumor necrosis factor receptor-associated periodic syndrome, IL-1Ra: Interleukin-1 receptor antagonist

**Table I.** Continued.

| P N | Fever | Abdominal pain | Skin rash | Artralgia /arthritis | Conjunctivitis | Family history | Pharangitis | Tonsillitis | Aphthous stomatitis | Adenitis | Headache | Myalgia | Hearing loss | Elevated acute phase reactans | Preliminary diagnosis | Final diagnosis | Treatment | Genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 35 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |
| 36 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 37 | + | + | - | +/- | - | + | - | - | - | - | + | + | - | + | uSAID | uSAID | C | Q703K/WT |
| 38 | + | - | + | +/- | + | - | - | - | - | - | - | + | - | + | uSAID | uSAID | - | Q703K/WT |
| 39 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 40 | + | + | + | +/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 41 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |
| 42 | + | - | - | +/- | + | - | - | - | + | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 43 | + | + | + | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 44 | + | - | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 45 | + | + | - | +/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | - | Q703K/WT |
| 46 | + | - | - | +/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 47 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | - | Q703K/WT |
| 48 | + | + | - | -/- | - | + | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 49 | - | + | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 50 | + | + | - | -/- | + | + | - | + | - | - | + | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 51 | + | + | - | +/- | - | + | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 52 | + | + | - | -/- | - | - | - | + | + | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 53 | + | + | - | -/- | - | - | - | - | - | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |
| 54 | + | - | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |
| 55 | + | - | - | -/- | - | - | - | - | - | - | - | - | - | + | uSAID | uSAID | C | Q703K/WT |
| 56 | + | - | - | -/- | - | - | - | - | - | - | - | - | - | - | uSAID | uSAID | C | Q703K/WT |

CAPS:Cryopyrin-associated periodic syndromes, HIDS: Hyperimmunoglobulin D Syndrome, PFAPA: periodic fever, aphthous stomatitis, pharyngitis and cervical adenitis, IVIG: intravenous immunoglobulin, NA: Not available, PN: Patient number, WT: Wild type, uSAID: Undefined systemic autoinflammatory disease, C: colchicine, P: Predinisolone, TRAPS: Tumor necrosis factor receptor-associated periodic syndrome, IL-1Ra: Interleukin-1 receptor antagonist

the remainder as uSAID (n=39). A flow chart illustrating the inclusion/exclusion process for patients is summarized in Supplementary Fig. 1. The main clinical findings, preliminary and final diagnosis of 56 patients with the Q703K mutation are summarized in Table I.

Among the 56 patients carrying the Q703K mutation, 18 were female (32.14%) and 38 (67.85 %) were male. The median age of the cohort was 10.3 (interquartile range (IQR): 7.06-12-98) years old. The median age of symptom onset was 36 (IQR:18-72) months old.

The common clinical findings are fever, abdominal pain, skin rash and musculoskeletal involvement (92.85%, 53.57%, 25 % and 25% respectively). All patients with musculoskeletal findings had only arthralgia and therefore they were classified as mild. Neurological findings (headaches only) were present in 6 (10.71%) patients. Ophthalmic findings (conjunctivitis)

were observed in 8 (14.28%) patients. None of the participants had hearing loss or amyloidosis. Lastly, 46.42% patients had elevated acute phase reactions.

After one year follow-up with their clinical findings and molecular genetic analysis results 10 patients were evaluated as CAPS mild phenotype. Of these patients two were treated with IL-1 inhibitors and responded well to treatment. An additional 7 patients were evaluated for their history of PFAPA syndrome. All 7 patients responded very well to colchicine treatment. The remaining 39 patients were classified with an uSAID (Supplementary Fig. 1).

## Discussion

In this study, we evaluated the clinical findings of 56 patients with the confirmed Q703K heterozygous variant. This patient cohort was identified through the pediatric rheumatology outpatient clinic, specifically for patients with autoinflammatory symptoms who underwent a PFS panel.

We detected the allele frequency of Q703K similar to Exome Aggregation Consortium (ExAC) data, and 1000 Genomes project in both the PFS and control groups (4.1, 5.1, 5.3, 4.8%, respectively). The ExAC database referred to the European/white population and the 1000G project referred to a mixed group of 60,706 subjects. The data from the Genome Aggregation Database (gnomAD) reported an allelic frequency of 3.8% and 5.1% in the general and European populations, respectively. The pathogenic effect and penetrance of the Q703K variant were not detected. The frequent detection of the Q703K variant in various cohorts has led to the investigation of the pathogenicity of this variant. The impact of the Q703K variant on NLRP3 protein function as well as the resulting phenotypic spectrum continues to be debated.

Theodoropoulou et al.[21] found the *NLRP3* Q703K variant to be significantly higher in patients with autoinflammatory disease compared to the gnomAD data, suggesting an association between this variant and CAPS, PFAFA and uSAID. However, they did not show the functional effect of this mutation on basal inflammatory activity. This study suggested that the risk of developing autoinflammatory disease is likely high in patients carrying this variant, but further studies are needed to obtain detailed information about severity and prognosis.

Vitale et al.[17] showed that patients carrying the *NLRP3* Q703K mutation may present with FCAS-like findings. However, they suggested that caution should be used in the interpretation of the mutation alone, in order to avoid overtreatment in the high frequency of healthy carriers.

While the Q703K variant is detected in a high frequency of healthy individuals, the carrier rate differs significantly across various ethnic groups, as seen in local studies. In particular, Aksentijevich et al.[12] found a 5% allele frequency in a healthy Caucasian group. In another study, the Q703K allele frequency was 8.4% in 130 healthy individuals of various ethnic backgrounds.[25] A third study randomly selected 806 individuals from Sweden and found the allele frequency to be 6.5%.[13] In our study, the allele frequency was 4.8% from the pool of 1341 healthy individuals.

In a 10-year multi-center study, 580 patients were examined for the *NLRP3* gene, following clinical suspicion of CAPS or other PFSs. Of these patients, 57 were found to carry the Q703K variant. The final diagnosis of 13 out of the 36 patients, who had both complete clinical data and genetic confirmation of the Q703K variant, was a PFS separate from CAPS. Additionally, 2 of the 36 patients carrying a Q703K mutation along with another *NLRP3* variant were diagnosed with CINCA and MWS. At the follow-up visit, the remaining 21 patients were reported to have mild clinical findings. Severe CAPS phenotype findings were not observed in any of the 36 patients. Moreover, most of the patients carrying the Q703K variant received

an alternative final diagnosis. As a result of this study, the authors considered the *NLRP3* Q703K variant to be a polymorphism without an evident functional or clinical effects.[26]

Lidar et al.[25] detected the *NLRP3* Q703K variant in 14 of 90 individuals who presented with autoinflammatory symptoms. Only 1 of these patients met the criteria for CAPS and responded well to treatment with IL-1 inhibitors. The Q703K allele frequency was similar in the patient and control groups (7.7% and 8.4%, respectively; p=0.85). The conclusion of the study supports that Q703K is a polymorphism rather than a disease-related mutation. Aksentijevich et al.[12] found a similar allele frequency of Q703K in their patient and control groups (4% and 5%, respectively; p=0.84). Based on these values, the authors concluded that the Q703K variant may in fact not be pathogenic.

When examining the plethora of available studies, we suspect that the Q703K variant is causing the inflammatory effect. Rieber et al.[27] reported that the secretion of NLRP3 inflammatory products (IL-1β, IL-18 and caspase 1) has similar activity in Q703K variant and healthy control groups. Based on this findings, Rieber et al.[27] concluded that symptomatic individuals with the Q703K variant may be experiencing such symptoms due to an underlying pathophysiology other than caspase-1 hyperactivation. Blomgran et al.[28] recently showed that delayed neutrophil apoptosis was not due to caspase-1 or IL-1 β activity, further demonstrating that there must be an alternative cause for the hyperinflammatory responses in the Q703K variants. In contrast, the Q703K variant has a low rate of complete response to anti-IL-1 treatment.

Verma et al.[13] describe a patient with CAPS phenotype and Q703K genotype who responded well to anti-IL-1 therapy. This patient's monocytes, as compared to that of 5 healthy controls, revealed high active IL-1 beta secretion and caspase 1 overactivation after lipopolysaccharide stimulation. This same group showed that the Q703K variant resulted in a gain-of-function mutation that subsequently led to an overactive NLRP3 inflammasome. They also reported that this variant has milder clinical findings.[16] After this study, with a note added to guidelines for genetic diagnosis and inherited recurrent fevers, it was recommended that this variant be considered a variant of uncertain significance (VUS) and that it should be reported by clinicians moving forward.[14]

In our study, we included 56 patients who came in for a clinical follow-up carrying Q703K allele. Of these 10 patients' final diagnosis were CAPS mild phenotype. Seven of these patients' preliminary diagnosis was CAPS according to the Federici score.[22] All patients had mild clinical symptoms and only 2 patients were using IL-1Ra (*interleukin-1 receptor antagonist)* therapy. Only one patient, whose preliminary diagnosis was CAPS according to Federici score was finally diagnosed as uSAID according to clinical follow-up. Although we found a high rate of Q703K positivity in the group in which CAPS was considered, we also found the Q703K variant in those who were not compatible with clinical CAPS. Specifically, the Q703K carrier rate within the control cohort (n=1461) which was evaluated at the genetic diagnosis center for other reasons (multiple congenital anomalies, severe growth retardation, etc.) was 4.8%. Although this situation was similar to previous studies, high Q703K positivity in patients with clinically suspected CAPS was remarkable.

In most studies, patients carrying the Q703K variant had a milder phenotype with no central nervous system (CNS) symptoms.[17,26,29] However, some recent studies have reported severe CNS manifestations and inflammation in individuals with the Q703K variant.[11,30] In our study, neurologic deficits were detected in 6 patients. Mild neurological findings (headache) were detected in 5 patients. Headache was observed in 2 of 10 patients diagnosed with CAPS. As a result of all these studies, it can be concluded that the inflammatory effect caused by Q703K is equivocal. Moreover, from our study as well as those conducted

across numerous other sites, the Q703K variant requires a special evaluation.

Because of a high family history, PFAFA syndrome seems to have a genetic background with a low penetrance.[20] In previous studies, MEFV, MVK, CARD15 genes, as well as NLRP3 gene, have been analyzed in PFAPA patients.[31-33] The effect of these genetic variants in PFAPA syndrome is not clearly known. Perko et al.[31] evaluated 62 PPAFA patients and detected a significant variant in the *NLRP3* gene 13 patients (21%) and the Q703K variant was detected in 9 of the PPAFA patients (14.5%) and was detected in 12 of 100 healthy individuals (12%). Thus, no significant difference was found between allele frequencies ($p>0.05$). They hypothesized that PFAPA may result from a combination of low-penetration variants with epigenetic and environmental factors. It has been reported that this variant may play a role in PFAPA pathogenesis by causing excessive NLRP3 inflammasome with a gain-of-function effect.[16] The role of the Q703K variant in the pathogenesis of PFAPA cannot be excluded due to a possible functional effect on inflammasome.

Our study is limited as no further genetic studies have been performed for whole exome sequencing and exclusion of somatic mutations. The small number of patients examined in our study limited the establishment of a genetic causal relationship. In our study, we found similar allele frequencies in the healthy group (141/1451, 4.8%) and the group with autoinflammatory symptoms (68/639, 5.3%) in line with previous studies.

Our study analyzed a cohort of patients to identify those carrying the Q703K variant. They were evaluated by the Eurofever/PRINTO classification criteria, which identified all carriers to have CAPS, within which, 10 patients met the CAPS diagnostic criteria. The final diagnoses were CAPS in 10 of 56 patients with Q703K variant (%17.85), a notably high percentage. Additionally, 7 patients' final diagnosis with this variant was PFAPA by evaluation of the Modified Marshall criteria. However, in the evaluation of a large number of control groups, the allele frequency in the Turkish population was found to be 4.8%. In the evaluation of the patients with autoinflammatory symptoms, the allele frequency was found to be 5.3%. The similar frequency across both groups (control and autoinflammatory groups) supports the hypothesis that Q703K is a polymorphism, however, its high incidence in patients with mild CAPS symptoms leads us to believe that this variant cannot be ignored. Furthermore, this mutation may be responsible for the phenotype when combined with other genetic variants or epigenetic alterations. As clinicians continue to see patients with autoinflammatory symptoms and/or the presence of the Q703K variant on genetic panels, we recommend reporting both the mutation and clinical evaluation of the patient to ensure proper diagnosis. This data will ultimately help solve the causative relationship between *NLRP3* Q703K variant and autoinflammatory diseases.

## Ethical approval

This study was approved by the local Ethics Committee of Health Science University, Education and Research Hospital (Approval Number: B.10.1.TKH.4.34.H.GP.0.01/07/26.01.2023). Informed consent was obtained from all individual participants' legal guardians included in the study.

## Author contribution

The authors confirm contribution to the paper as fallows: study conception and design: YKD, BS; data collection: YKD, FD; analysis and interpretation of results: YKD; draft manuscript preparation: YKD, LAJ. All authors reviewed the results and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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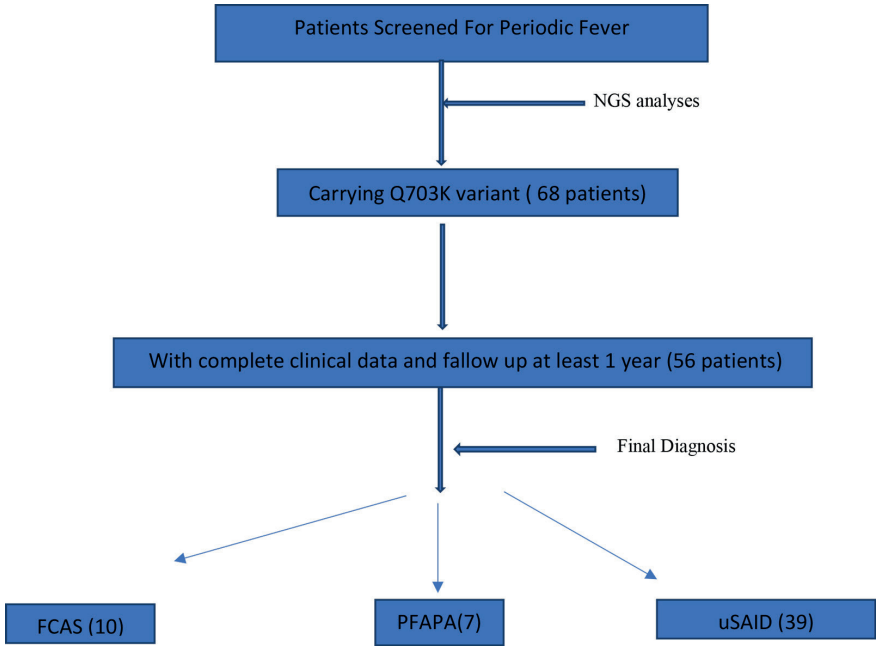


**Supplementary Fig. 1.** Flow-chart of the patients.

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Original Article

# The association of meatal stenosis and infant circumcision

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## ABSTRACT

**Background.** The association of meatal stenosis with age at circumcision is controversial. We noticed a high rate of meatal stenosis in a region where early circumcision is traditional. The aim of this study is to compare the age at circumcision between boys with or without meatal stenosis.

**Methods.** After ethical approval, families of children with meatal stenosis were questioned about age at circumcision and reason for circumcision. Control group consisted of patients with diagnoses other than penile abnormalities, a normal urethral meatus, and having no symptoms about urination. Patients with a history of therapeutic circumcision were excluded from the study.

**Results.** Between November 2016 and November 2020, 115 patients with meatal stenosis were admitted. All were corrected with ventral meatotomy under general anesthesia. Median age at circumcision was 3 (min:0-max:111) monthsand age at admission was 74 (min:22-max:194) months. Control group consisted of 205 boys. Median age at circumcision was 5 (min:0-max:122) months and age at admission was 96 (13-202) months. There was a statistically significant difference between groups in terms of age at circumcision (p=0.024) but none for age at admission (p=0.356). There was a twofold increase in the meatal stenosis rate (39% vs. 23%) if circumcision was performed before age one (p=0.018). There was no difference between the patients circumcised in the newborn period and later (38% vs 36%, p=0.778).

**Conclusions.** Our study supports the previous reports suggesting a relation of risk for meatal stenosis and age at circumcision and presents data that age one might be a cutoff for this risk.

**Key words:** circumcision, infant, neonatal, meatal stenosis, meatal web.

American Academy of Pediatrics (AAP) changed its policy statement on circumcision in 2012, and specified that the benefits of circumcision in the newborn period are sufficient to justify leaving the decision to the parents but not great enough to recommend routine circumcision for all male newborns.[1] Since then, infant circumcision has preserved its popularity.

Meatal stenosis is one of the common complications of circumcision.[2] Meatal stenosis rate is higher in circumcised males[3,4] but the association of meatal stenosis with age at circumcision is controversial. We noticed an extraordinarily high rate of meatal stenosis in a region where early circumcision is traditional. The aim of this study is to compare the age at circumcision between boys with or without meatal stenosis.

## Material and Methods

The study was mainly based on retrospective inquiry of the parents, but also included physical examination and voiding observation of the children. The inquiry consisted of two short questions as age at circumcision and the reason for circumcision.

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The study group included patients operated due to urethral meatal stenosis between November 2016 and November 2020. The diagnosis of meatal stenosis was made observing the prolonged and upward directed urinary stream with narrow caliber.[5] All patients with meatal stenosis were treated with meatotomy. Meatotomy was performed under general anesthesia and involved incision and suturing of the web on the ventral aspect of the meatus while calibrating the urethra using a stent with a diameter appropriate for age. None of the authors prefer dilations for meatal stenosis as they think it does not provide a permanent solution.

The control group was constituted from circumcised boys who were admitted to the same hospital between November 2019 and November 2020. These were patients with diagnoses other than penile abnormalities, having a normal-looking urethral meatus, and reporting no symptoms about urination. After informed consent, their parents were inquired about age at and reason for circumcision. Normal urine flow was confirmed with videos of voiding in the control group. Patients with a history of therapeutic circumcision and the ones with a duration less than one year since circumcision were excluded from the study.

This study was conducted at a 665 bed, secondary care children's referral hospital with approximately 485.000 patient admissions annually. The city where the study was performed is in south-east of Türkiye where early circumcision (after the 40$^{th}$ day of life) is traditional but not consistently performed by all. Ethical approval was obtained from the local ethics committee (Gaziantep University, Ethical Board for Clinical Studies, decision number: 2019/287). Written informed consent was obtained both from the parents and children.

The statistical analyses were performed using IBM SPSS Package Version 25 (Armonk, NY, USA: IBM Corp.). Histograms and the Kolmogorov-Smirnov test were performed to check the normality of distribution of the continuous variables. Descriptive statistics were used to summarize patient characteristics. A p-value below 0.05 was considered statistically significant. The Mann-Whitney U test was used to compare age at circumcision and age at admission between the patients with or without meatal stenosis. Pearson Chi-square test was used to evaluate the difference in meatal stenosis rate between subgroups according to age at circumcision. A power analysis using G*power 3 software was also performed.[6]

## Results

In total, 115 patients with meatal stenosis were admitted to our hospital during the study period. All were corrected with ventral meatotomy under general anesthesia, and all accepted to be included in the study. There were 205 participants in the control group. Main characteristics of the study group are present in Table I. There was a statistically significant difference between groups in terms of age at circumcision (p=0.024) but none for age at admission (p=0.356). There was also no difference between groups regarding duration between the circumcision and admission (p=0.141).

We then evaluated how meatal stenosis ratio changed in our study group regarding circumcision at different specific time periods such as during infancy, during newborn period, before the end of mini puberty, or during mini puberty (Table II). There was no difference between the patients circumcised in the newborn period and later (38% vs 36%, p=0.778). Meatal stenosis rate seemed to be higher in the first six months (Fig. 1), but the most prominent cut-off was at age 1 years. The number of patients with meatal stenosis was almost double when patients who had circumcision before age one or later were compared (39% vs. 23%) (p=0.018). There was also a statistically significant difference between the patients circumcised before or after 6 months (42% vs 27%, p=0.008) but none

**Table I.** Main characteristics of the study group.

| | Number of patients | Age at circumcision | Age at admission | Duration between circumcision and admission |
|---|---|---|---|---|
| Patient Group | 115 | 3 (0-111)* | 75 (22-194)* | 71 (7-182)* |
| Control Group | 205 | 5 (0-122)* | 96 (13-202)* | 81 (12-177)* |
| | | p=0.024 | p=0.356 | p=0.141 |

*depicted as months; median (range).

**Table II.** Number of subjects with meatal stenosis in different time limits.

| Number (%) of subjects | Number (%) of subjects | | |
|---|---|---|---|
| | with meatal stenosis | without meatal stenosis | p |
| when newborn | 26 (38%) | 43 (62%) | 0.778 |
| later | 89 (36%) | 162 (64%) | |
| first 6 months | 83 (42%) | 117 (58%) | 0.008 |
| later | 32 (27%) | 88 (73%) | |
| first 12 months | 101 (39%) | 157 (61%) | 0.018 |
| later | 14 (23%) | 48 (77%) | |
| between 0-6 months | 83 (42%) | 117 (58%) | 0.171 |
| between 6-12 months | 18 (31%) | 40 (69%) | |
| during mini-puberty (2-6 months) | 57 (44%) | 74 (56%) | 0.024 |
| any other time | 58 (31%) | 131 (69%) | |

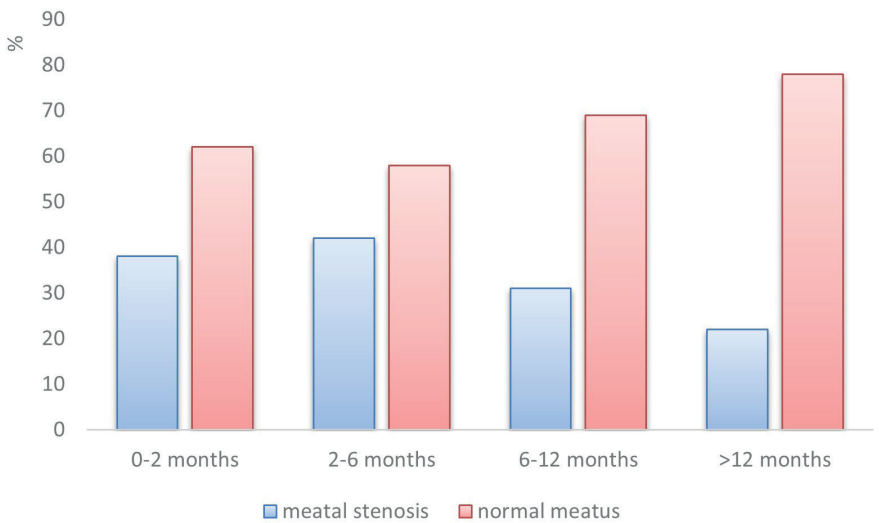


**Fig. 1.** The percentage of patients with meatal stenosis for each age group.

between patients circumcised in the first six months and between 6 to 12 months (42% vs 31%, p=0.171). We also evaluated circumcisions during mini puberty (2-6 months) or another time (including the newborn period), and saw a similar finding (44% vs 31%, p=0.024). Overall, the ratio of patients with meatal stenosis seemed to be similar until age one years, and significantly smaller after then.

## Discussion

Circumcision is one of the oldest and most commonly performed procedures but controversy on its necessity and when to perform it continues.[7] Our study focuses on the association of meatal stenosis and age at circumcision. The advantage of circumcision regarding decreased risk of urinary infections is more prominent when performed in the newborn period.[8] On the other hand, the prepuce cannot be retracted fully in 96% of infants[9] which necessitates forceful retraction of the prepuce during circumcision in the newborn period. Exposure of delicate mucosa to ammonium or mechanical trauma in a child with diapers, ischemia of the meatal mucosa stemming from damage to the frenular artery, and forceful degloving of the preputium have all been implicated in the etiology of meatal stenosis.[5,10,11] Therefore, an association between meatal stenosis and age at circumcision seems straightforward.

One problem while discussing meatal stenosis is its definition. The rate of meatal stenosis is highly varying among papers.[3,11] A metanalysis reported data supporting increased risk of meatal stenosis following circumcision (with an odds ratio of 3.20) but that overall rate is low (<1%).[3] On the other hand, a large retrospective series on infant circumcision revealed that one fourth of the revision surgeries were due to meatal stenosis after neonatal circumcision.[12] This is probably due to the differences in its definition.

Özen et al.[13] reported that patients admitted as meatal stenosis following infant circumcision had a web-like structure on the ventral aspect of the meatus. Therefore, they suggested using the name "meatal web" instead of "meatal stenosis". We agree with their observation regarding the anatomy of the pathology but preferred to use the common nomenclature as their proposal did not find widespread usage. Lichen sclerosus is also associated with meatal stenosis[14] which is probably a different entity. And as discussed, the effect of inflammation on meatal stenosis is also debated. Therefore, we excluded all cases who underwent a therapeutic circumcision to understand the relationship with age solely.

Several large-scale studies showed circumcision increases the risk of meatal stenosis[3], but scarce studies addressed its relation with age at circumcision and the threshold is varying. A prospective cohort study on 1100 participants by Howe[15] showed that all children with meatal stenosis were circumcised neonatally. Acimi et al.[16] showed a twofold increase when comparing first week and that between 7-12 months. Likewise, Machmouchi et al.[11] found a higher rate of meatal deformity (reminding our definition of meatal stenosis) comparing the neonatal period with 5 months (90% vs. 11%). We found that age at circumcision was significantly smaller in children with meatal stenosis but unlike previous studies, there was no difference in the ratio of patients with meatal stenosis between the newborn period and later. It changed significantly at age one. We also searched for a specific relationship about circumcision during or before the completion of mini puberty, but the rate was similar until age 1 years with no particular association with mini-puberty. Our results may support the retractability theory for the etiology of meatal stenosis following circumcision as retractability rate of the prepuce increases to 50% at age one years[9] but more data is obviously required to draw clearer conclusions.

The major limitation of our study is not demonstrating an overall meatal stenosis prevalence in the population it was performed. But our study does not make any claims regarding the prevalence or etiology of meatal stenosis. Another problem is that the majority of the patients were circumcised in infancy, which we think can also explain the high number of patients with meatal stenosis in a study involving patients in a four-year period. Also, we do not know when meatal stenosis exactly happens, therefore we do not know if the participants in the control group will experience meatal stenosis later. Participants who were admitted

in the year following circumcision were excluded from the study to overcome this bias. Another limitation is that our study involves no data about the technique of circumcision, postoperative care after the circumcision or preservation of frenulum which can also be contributive factors for meatal stenosis. Besides these limitations of a retrospective study, the data retrieved is retrospective but what we ask the parents is their child's age at circumcision and if it was due to therapeutic reasons, which we think are not questions open to recall bias.

As mentioned above, meatal stenosis definition and rate differed significantly among the published papers, so a proper estimation of sample size was also impossible. Minimum sample size for each group had to be somewhere between 4 and 7178 according to published papers.[3,11] Therefore, we aimed to include all patients with meatal stenosis and a higher number of participants in the control group (circumcised patients with no meatal stenosis). Then, we performed a post-hoc power analysis. The power of our study to detect the difference between meatal stenosis rate in patients who underwent circumcision before or after age one with a 5% level of significance was calculated as 97%.

American Association of Pediatrics leaves the decision of infant circumcision to the parents and the responsibility of informing them about its advantages and disadvantages to their physicians.[1] The discussion with parents is mainly based on the risk of UTI or the rare catastrophic complications of circumcision. The risk for meatal stenosis is seldom discussed in detail with parents. We think it's important for a parent to know that circumcision can result in one more intervention and this actually might be associated with the age it was performed.

Our study supports previous reports suggesting a relation of risk for meatal stenosis and age at circumcision and presents data that age one years might be a cutoff for this risk. Further studies are required to investigate this association, and families should be informed about the risk of meatal stenosis while discussing timing of circumcision.

## Ethical approval

Ethical approval was obtained from the local ethics committee (Gaziantep University, Ethical Board for Clinical Studies, decision number: 2019/287). Written informed consent was obtained both from the parents and children.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ST; data collection: ST, YI; analysis and interpretation of results: ST, YI; draft manuscript preparation: ST, YI. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

## Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

# Fatal thrombotic microangiopathy in an infant with COVID-19: a case report

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## ABSTRACT

**Background.** While macrovascular thrombosis is common in adult COVID-19 patients, thrombotic microangiopathy as a part of endothelitis might play an important role in severe organ dysfunction. Thrombocytopenia-associated multiple organ failure (TAMOF) is a thrombotic microangiopathy syndrome that is associated with endothelial damage. Herein, we aim to report a pediatric TAMOF case related to SARS-CoV-2 infection which has been scarcely reported to date.

**Case.** A 7-month-old boy who became severely ill after being infected with SARS-CoV-2 required advanced critical care treatments such as continuous renal replacement therapy, therapeutic plasma exchange, and extracorporeal membrane oxygenation. A heart and lung biopsy obtained during sternotomy showed thrombotic microangiopathy. Despite early plasma exchange, mortality was inevitable because of severe liver failure.

**Conclusions.** This case report implies that SARS-CoV-2 infection could cause TAMOF in children. To the best of our knowledge, this is the second SARS-CoV-2-induced pediatric TAMOF case. More studies are needed to determine alternative treatments for patients with TAMOF who are resistant to conventional therapies.

**Key words:** COVID-19, TAMOF, thrombotic microangiopathy, therapeutic plasma exchange.

Thrombocytopenia-associated multiple organ failure (TAMOF) is a thrombotic microangiopathy syndrome that is associated with endothelial damage caused mostly by infections. TAMOF results from immune dysregulation and impaired A Disintegrin And Metalloproteinase with ThromboSpondin type 1 motif member 13 (ADAMTS13) activity. TAMOF is characterized by new onset thrombocytopenia and progression to at least two organ system failures. Von Willebrand factor (vWF) and ADAMTS-13 (or vWF-cleaving protease) play a central role in TAMOF. Herein, we aimed to report a TAMOF case related to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) infection and coronavirus disease 2019 (COVID-19).

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## Case

A 7-month-old boy was brought to the emergency department with vomiting, diarrhea, decreased urine output, and respiratory distress. He was born to nonconsanguineous parents in the 35$^{th}$ week of pregnancy with a history of polyhydramnios and was diagnosed with anal and esophageal atresia, and an H-type tracheoesophageal fistula. Shortly after birth, he had repair surgery (resection of the fistula and end-to-end anastomosis of the esophageal blind sides) and a colostomy for anal atresia. He was intubated for 4 days, suffered from sepsis due to *Klebsiella pneumoniae*, pleural effusion requiring drainage, and was discharged after 2 months. He had thrived well since then; however, he visited the hospital for vomiting and decreased

weight gain due to gastroesophageal reflux one week before. On physical examination, fever, tachycardia, tachypnea, severe dehydration, weak pulses, prolonged capillary refill time, generalized hypotonia, impaired consciousness, and intercostal retractions were profound. After three fluid boluses, he needed adrenaline infusions and was intubated for severe respiratory distress and hypoxia. Both nasopharyngeal swabs and deep tracheal aspirates were positive for SARS-CoV-2, (England variant). Chest X-ray showed bilateral patchy infiltration. Primary laboratory data showed mild metabolic acidosis, increased lactate level (10 mmol/L), leukopenia (2,900 cells/mm$^3$), and neutropenia (270 cells/mm$^3$) (Table I).

**Table I.** Laboratory findings of the patient.

| Laboratory parameter | Baseline (1 week before) | At presentation | Before TPE | Day 3 | Day 6 | Day 8 | Day 12 |
|---|---|---|---|---|---|---|---|
| pH | | 7.27 | 7.36 | 7.38 | 7.19 | 7.22 | 7.34 |
| $pCO_2$ (mmHg) | | 53.3 | 45.6 | 50.7 | 48.6 | 40.7 | 44.9 |
| $HCO_3$ (mmol/L) | | 21.5 | 23.6 | 27.8 | 16.5 | 16.0 | 22.8 |
| Base excess (mmol/L) | | -2.7 | 0.7 | 5.2 | -9.8 | -10.8 | -1.2 |
| Lactate (mmol/L) | | 1.6 | 1.5 | 1.3 | 7.4 | 17 | 7.1 |
| White blood cell (/mm$^3$) | 13,100 | 2,900 | 3,900 | 11,400 | 4,700 | 6,100 | 6,100 |
| Lymphocyte (/mm$^3$) | 7,950 | 2,100 | 1,700 | 790 | 570 | 100 | 420 |
| Neutrophil (/mm$^3$) | 2,460 | 270 | 2,000 | 10,360 | 4,000 | 3,650 | 5,420 |
| Platelet (/mm$^3$) | 419,000 | 295,000 | 62,000 | 147,000 | 28,000 | 23,000 | 66,000 |
| Hemoglobin (g/dL) | 12.5 | 12.3 | 11.3 | 11.2 | 11.4 | 14.3 | 12.2 |
| ALT (U/L) | 16 | 35 | 80 | 105 | 866 | 4,134 | 66 |
| AST (U/L) | 36 | 190 | 245 | 341 | 1,456 | 3,479 | 887 |
| CK (U/L) | 27 | 150 | 367 | 350 | 250 | | 365 |
| GGT (U/L) | 20 | 25 | 27 | 17 | 134 | 270 | 165 |
| ALP (U/L) | 223 | 161 | 117 | 92 | 69 | 176 | 157 |
| Albumin (gr/dL) | 3.4 | 2.8 | 2.27 | 2.68 | 2.89 | 2.77 | 2.86 |
| Total bilirubin (mg/dL) | 0.25 | 0.21 | 0.35 | 0.17 | 1.11 | 4.26 | 12.3 |
| Direct bilirubin (mg/dL) | 0.04 | 0.128 | 0.07 | 0.05 | 0.71 | 1.89 | 6.9 |
| INR | 1.01 | 1.75 | 1.75 | 1.35 | 4.0 | 2.94 | 2.9 |
| Sodium (mEq/L) | 135 | 142 | 142 | 143 | 138 | 138 | 138 |
| Potassium (mEq/L) | 3.68 | 2.99 | 3.26 | 3.39 | 4.15 | 3.15 | 3.63 |
| Calcium (mg/dL), corrected | 10.6 | 8.4 | 8.2 | 8.9 | 8.6 | 10.3 | 10.6 |
| Phosphorus (mg/dL) | 4.97 | 6.59 | 5.74 | 2.41 | 3.37 | 3.3 | 1.83 |
| Creatinine (mg/dL) | 0.23 | 0.89 | 0.70 | 0.55 | 0.95 | 0.64 | 0.29 |
| Blood urea nitrogen (mg/dL) | 14.7 | 53.3 | 46.5 | 25.3 | 28.4 | 11.0 | 2.8 |
| Uric acid (mg/dL) | 4.58 | 17.8 | 11.97 | 7.93 | 5.98 | 6.89 | 0.43 |
| Troponin-I (ng/L) | - | 236 | 190 | | | | |
| Ferritin (μg/L) | - | 1,001 | 1,237 | 110 | 1,850 | 35,910 | 660 |
| Interleukin-6 (pg/mL) | - | 6,525 | 7,291 | 1,156 | 2,816 | 2,814 | 75.6 |
| C-reactive protein (mg/dL) | - | 2.41 | | | | 2.89 | 2.19 |
| Procalcitonin (ng/mL) | - | 246 | 352 | 12.4 | 90 | | |

ALP: alkaline phosphatase, ALT: alanine aminotransferase, AST: aspartate aminotransferase, CK: creatine kinase, GGT: gama-glutamyltransferase, INR: international normalized ratio, TPE: therapeutic plasma exchange.

Although platelet count was in the normal range (295,000 cells/mm$^3$), it decreased from 419,000 cells/mm$^3$ during his last routine visit six days ago. The pediatric risk of mortality (PRISM-III) score was 22, probability of death rate was 26%. Multiorgan dysfunction syndrome score was 9 and the pediatric logistic organ dysfunction (PELOD) score was 32 on admission day. Echocardiography was normal (75% of the ejection fraction). Because thrombocytopenia was accompanied by dysfunction of two major organ systems, the diagnosis was TAMOF. He was treated with therapeutic plasma exchange (TPE) three times. TPE was suspended because the PELOD score decreased to 2 and platelet count increased to 147,000 cells/mm$^3$. On the 6$^{th}$ day, he was extubated with non-invasive ventilation. However, within 24 hours' acute respiratory distress syndrome (ARDS) occurred accompanied by severe cardiovascular failure so he underwent central venoarterial (right atrium-aorta) extracorporeal membrane oxygenation (ECMO) with a centrifugal pump and pediatric oxygenator (Liliput 2, Sorin group™). Central cannulation was chosen according to the experiences of the surgical team and available equipment. During sternotomy for ECMO cannulation, with the family's permission, heart, and lung biopsies were obtained to better understand the pathophysiological processes of SARS-CoV-2 infection in children. It revealed increased alveolar inflammatory cells and thrombotic microangiopathic changes in the small vessels of the alveoli. Myocardial edema and differences in the volumes and shapes of myofibers but no inflammatory cells were detected on myocardial biopsy. He received broad-spectrum antibiotics which were tapered later because no bacterial or fungal organisms were detected on sequential blood and other body fluid cultures. Daily TPE with 1.5 times of plasma volume was performed for TAMOF with significant liver failure (maximum ALT level was 3,450 U/L). Intravenous immunoglobulin (IVIG) (0.4 gr/kg, 5 days) was given between TPE sessions as in the Zipper method of Hacettepe.[1] Levosimendan infusion was started right after ECMO initiation because of significantly impaired left ventricular dysfunction requiring full cardiac support (120 ml/kg of blood flow rate). Continuous venovenous hemodiafiltration was started through the ECMO circuit for hypervolemia and renal failure. Because the ferritin, interleukin-6 and procalcitonin levels peaked for the second time anakinra (1 mg/kg, 2 doses) was added. The maximum ferritin level was recorded as 35,910 μg/L (Fig. 1) just before initiating anakinra and levosimendan. He showed a good response to the combination of TPE, IVIG, and anakinra treatment around the 12$^{th}$ day. However, he lost his brainstem reflexes on the 14$^{th}$ day and died the next day (Fig.1). Written informed consent was obtained from the parents for this case report.

## Discussion

Approximately 80% of children infected with SARS-CoV-2 develop mild to moderate disease and the incidence of critical illness is high in children under 1 year old.[2] Herein, we report a fatal TAMOF case associated with SARS-CoV-2. TAMOF is characterized by severe organ dysfunction and often new onset thrombocytopenia. The common pathophysiology of thrombotic microangiopathies is systemic endothelial injury. Abnormally large vWF multimers induce platelet aggregation resulting micro-thrombi in vessels that cause organ damage.[3] Endothelial dysfunction is also an important feature of SARS-CoV-2 infection.[4] Extensive micro-thrombosis promoted and aggravated by endothelial dysfunction which might be the result of direct viral effects and/or systemic inflammation could explain the profound elevation of D-dimers and thrombocytopenia in severe COVID-19.[5] Although ACE2 expression and other endothelial biomarkers are the hallmark of both pulmonary and non-pulmonary pathology of COVID-19, Mancini et al.[6] showed a quantitative imbalance between the vWF and ADAMTS13, with a seven-fold increased vWF antigen to ADAMTS13 activity ratio associated with severe COVID-19 that required intensive care and mechanical ventilation.

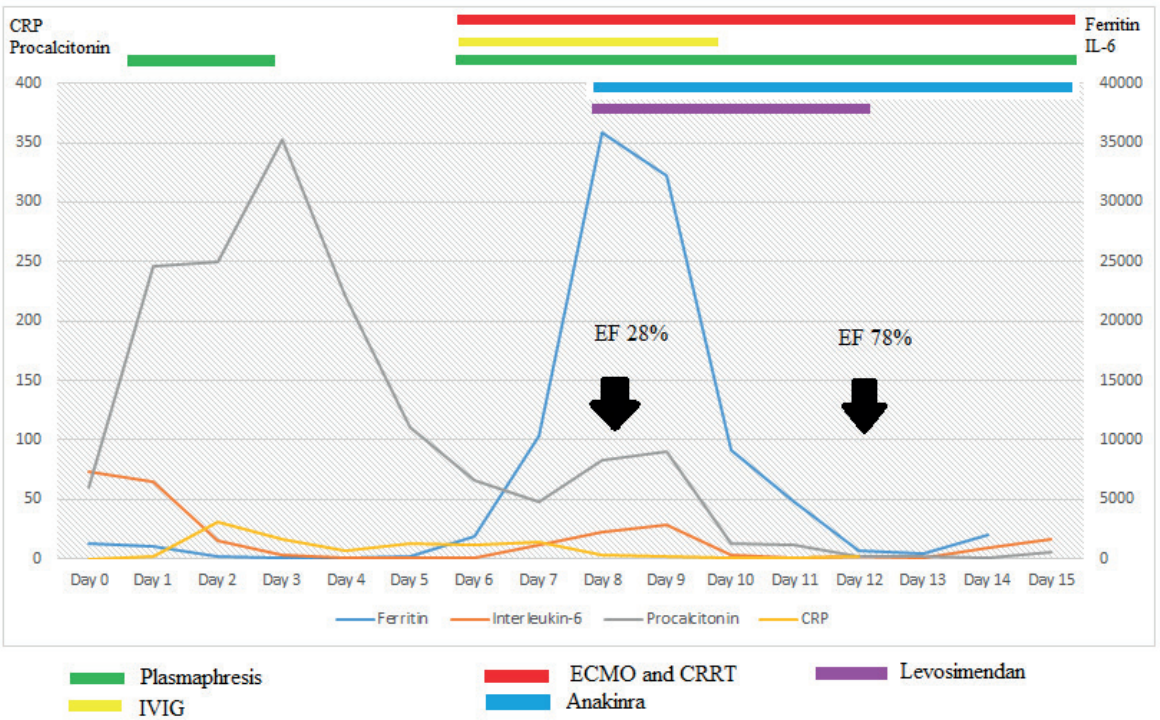


**Fig. 1.** The chronological interventions to the patient. Levels of ferritin are shown in μg/L, Interleukin-6 in pg/mL, CRP in mg/dL and procalcitonin in ng/mL.

We did not have the chance to study ADAMTS13 activity or vWF antigen levels in our patient. However, severe organ dysfunctions and new onset thrombocytopenia with evidence of acute SARS-CoV-2 infection led us to treat the patient as COVID-19-related TAMOF. The diagnosis of TAMOF was later supported by microangiopathy findings in the pathological specimen. Thrombotic microangiopathy and its relation to COVID-19 is well-defined in adults, however, few pediatric cases have been reported to date.[7,8] Latimer et al.[7] reported a patient who survived intensive treatment. Our patient improved after three days though a severe cytokine storm was the main reason for his worsening. After penetrating respiratory epithelial cells, SARS-CoV-2 triggers an immune response with proinflammatory cytokine production due to the rapid activation of Th1 cells. By the infiltration of macrophages and neutrophils into the lung tissue, which results in a cytokine storm.[9] Although multiple TPE, anakinra, IVIG, and steroid treatments alleviated the cytokine storm and levosimendan infusion corrected the left ventricular function (Fig.1), the liver failure had become the main determinative factor for death. Severe liver failure resulting from cytokine storm might have contributed to brain damage although ongoing treatment of plasmapheresis. Direct myocardial invasion of the virus can cause myocarditis and death.[10] That our patient's myocardial biopsy showed no viral particle, but myocardial edema and elevated serum inflammatory markers suggested multisystemic involvement.

Daily plasma exchange until thrombocytopenia reverses can restore the ADAMTS13 and other coagulation factors and improve the organ failures in TAMOF.[11] It also has the advantage of removing proinflammatory cytokines. The fresh frozen plasma acquired from the patients who recovered from COVID-19, called convalescent plasma, used to treat COVID-19 patients in active phase of infection. The specific antibodies in convalescent plasma help the patient fight against the virus.[12] Latimer et al.[7] have reported a similar pediatric case that resolved after two

sessions of plasma exchange and aggressive supportive care. They explained that the reason for the avoidance of multiple plasmapheresis was the concern of the clearance of antibodies against SARS-CoV-2. Unlike them, we carried out multiple plasmapheresis because of severe hepatic failure.

To the best of our knowledge, this is the second SARS-CoV-2 induced pediatric TAMOF case. Although COVID-19 causes a mild clinical phenotype in children, TAMOF should be considered in patients with severe organ dysfunction and new onset thrombocytopenia.

## Ethical approval

Written informed consent was obtained from the parents for this case report.

## Author contribution

The authors confirm their contribution to the paper as follows: study conception and design: BB, SK; data collection: ÖSN; analysis and interpretation of results: ÖSN, KT; draft manuscript preparation: ÖSN, SK. All authors reviewed the results and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

# Multidisciplinary treatment for acute massive upper gastrointestinal bleeding secondary to post-burn stress in a paediatric patient: a case report

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## ABSTRACT

**Background.** Severe burns can readily induce gastric and duodenal mucosal erosions and superficial ulcers. In severe cases, haemorrhage or perforation of peptic ulcers might occur, threatening the lives of patients. At present, gastrointestinal haemorrhage after burns is treated mainly with drugs and gastrointestinal endoscopy. However, multidisciplinary treatment of gastroscopy combined with vascular embolization is rare.

**Case.** A boy aged 3 years and 4 months was admitted to the hospital, scalded by boiling water on multiple parts of the body. On the 8th day after the injury, the patient continuously produced a large amount of tarry black stool, and the faecal occult blood test was positive. Haemostatic drug treatment was ineffective, and severe shock and disseminated intravascular coagulation (DIC) occurred. Under the guidance of a multidisciplinary team (MDT), a gastroscopy examination was performed and showed bleeding from a duodenal bulb ulcer. Due to a small intestinal lumen and thin intestinal wall, bleeding could not be controlled by gastroscopy. However, the bleeding point was clarified by gastroscopy and then gastroduodenal artery embolization was performed efficiently. No active gastrointestinal bleeding was observed after the surgery. The patient was followed for 6 months after discharge, and no gastrointestinal haemorrhage recurred.

**Conclusions.** This is a rare case of acute massive upper gastrointestinal bleeding secondary to post-burn stress in paediatric patients. For paediatric patients who cannot be treated by endoscopy, transcatheter embolization may be safer and more effective for achieving haemostasis. Through the collaboration of the MDT, gastroscopy combined with interventional embolization was performed, which successfully stopped the massive bleeding and saved the child's life, making it worthy of clinical reference.

**Key words:** curling ulcer, upper gastrointestinal bleeding, gastroscopy treatment, vascular embolization.

Severe burns can readily induce gastric and duodenal mucosal erosion or ulceration due to intense stress. Postburn stress ulcer, also known as Curling's ulcer, is a digestive system complication that occurs after severe burns. In addition to gastrointestinal mucosal oedema, congestion, bleeding and erosion, severe gastrointestinal bleeding ulcers or perforations can occur, which directly threatens the patient's life.[1] The incidence of gastrointestinal ulcers combined with bleeding is approximately 4.7%, but in most cases, they are actually a diffuse oozing of blood in the intestinal cavity. At present, acute massive hemorrhage from gastrointestinal ulcers in burn patients is rare. A previous study has shown a 50% mortality rate observed in patients with ulcers/hemorrhages within 27 days of admission.[2] It is obvious that burns in pediatric patients are more dangerous and burdened with a higher risk of complications than burns in adults.[3] For the active treatment of the primary disease and

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the elimination of stress factors, sedation, anti-shock, acid suppression and pharmacological haemostasis are mainly used[4], and the use of endoscopy combined with interventional embolization for haemostasis is extremely rare. A case report of the diagnosis and treatment of acute upper gastrointestinal haemorrhage in a paediatric burn patient is reported.

## Case Report

### *1. Admission*

The patient was 3 years and 4 months old. The patient was accidentally scalded on the torso, both lower limbs, the left upper limb and the buttocks by boiling water at home 4 days prior to presentation. Immediately after the injury, the wound was treated with Chinese herb medicine powder at the local clinic (the specific formula that was used is unknown), and the patient was admitted to our hospital due to aggravation of his condition. At the time of admission, the patient was in poor general condition. Physical examination showed that the burn wounds were distributed on the trunk, both lower limbs, the left upper limb and the buttocks, covering a total area of approximately 9% (Fig. 1). Most of the wounds were covered with dark brown eschar, and the area surrounding the wounds was red and swollen. Diagnosis on admission indicated that the depth of burns was 3rd degree with 9% total burn surface area (TBSA). The patient had no previous systemic diseases, including congenital heart disease or gastrointestinal ulcer.

### *2. Diagnosis and treatment after admission*

After admission, the patient was treated with intensive care, anti-infection treatment, acid suppression, correction of hypoproteinaemia, maintenance of the stability of the internal environment and wound dressing changes. The electrocardiogram (ECG) and chest X-ray showed no abnormalities at the time of admission. Procalcitonin (PCT) was 11.57 ng/mL, the white blood cell count (WBC) was 9.64×10^9/L, the neutrophil percentage (Neu%) was 76.00% and C-reactive protein (CRP, colloidal gold method) was >200.00 mg/L. The increase in infection indicators in the patient may have been caused by the absorption of toxins from the burn wounds. To remove necrotic tissue in a timely manner and control the infection, wound eschar removal + autologous skin transplantation was performed on the 7th day after injury. Degenerative necrosis of the adipose tissue under the eschar was observed during surgery, and some wounds reached the deep fascia and muscle tissues. The operative area was 9%, and the duration of the operation was 2 hours and 25 minutes. Intraoperative blood loss was approximately 350 ml, and 400 ml of red blood cell suspension and 180 ml of plasma were transfused. Intraoperative vital signs were relatively stable, and postoperative rehydration, haemostasis and acid suppression treatments were performed.

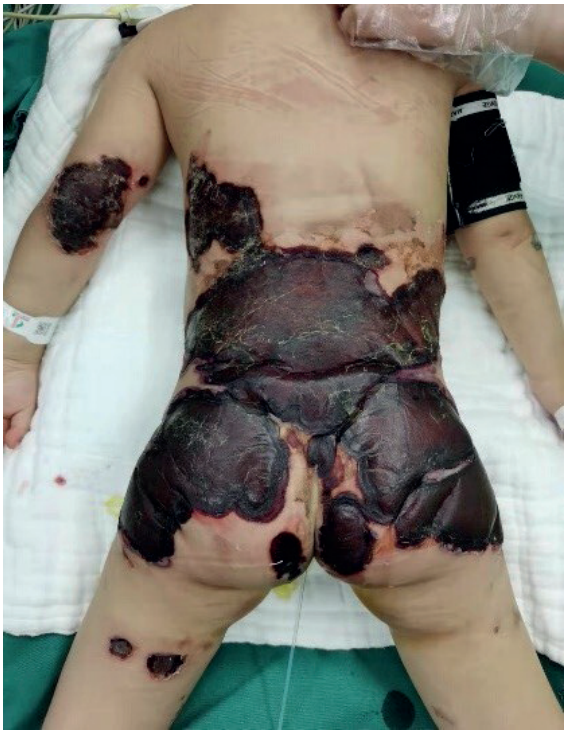

**Fig. 1.** The patient's wound at the time of admission.

### *3. Diagnosis and treatment of gastrointestinal bleeding*

At 07:05 on postoperative Day 1, the patient was found to have tarry black stool, with a volume of approximately 100 ml. Given the bleeding tendency of gastrointestinal stress ulcers, the intravenous push administration of haemocoagulase was immediately used to stop the bleeding, and 200 ml of red blood cell suspension was transfused. An emergency complete blood count (CBC) showed a red blood cell count (RBC) of 1.79×10^12/L, haemoglobin (HGB) of 48 g/L, haematocrit (HCT) of 13.60% and a platelet count (PLT) of 39×10^9/L. After an emergency multidisciplinary team (MDT) consultation was conducted with the departments of paediatrics, gastroenterology and haematology, the patient was diagnosed with upper gastrointestinal bleeding. The patient was given intravenous haemagglutinin, an intramuscular injection of vitamin K1, a gastric tube injection of norepinephrine with ice-cold saline, esomeprazole acid suppression, somatostatin, fluid infusion and blood transfusion to prevent shock, but the patient continued to produce a large amount of tarry black stool, his blood pressure dropped to 80/35 mmHg, and his heart rate increased to 165 beats/min despite continuous massive rehydration and supplementation with blood products. Repeated CBC suggested that HGB and platelets still showed a progressive decline. Due to persistent gastrointestinal bleeding, the treatment effect was not sufficient, and treatment was difficult. Therefore, the MDT collaborated again in cooperation with the departments of gastroenterology, vascular surgery, paediatrics, critical care medicine, general surgery and blood transfusion to develop a stepwise treatment regimen. The following regimen was used: 1. Rehydration and anti-shock therapy to maintain blood pressure; 2. Emergency gastroscopy and haemostasis; 3. If the haemorrhage could not be stopped under gastroscopy, vascular embolization could be considered; 4. If gastroscopy and interventional haemostasis failed, a surgical treatment plan of open abdominal exploration + resection of the bleeding gastrointestinal segment would be considered.

Gastroscopy (OLYMPUS GIF-Q260J; Endoscope outer diameter 9.9mm, Endoscope inside diameter 3.2mm; Image processing system CV-260SL) revealed active bleeding in the duodenal bulb. Due to the patient's small intestinal cavity, it was difficult for the fibre-optic cable of the gastroscope at our hospital to penetrate the duodenal bulb to stop the bleeding, and there was a risk of repeated damage to the intestinal wall. Due to the possibility of heavy bleeding, the regimen of haemostasis under gastroscopy was abandoned. At this time, the patient's condition further deteriorated. Blood gas analysis showed that the pH was <6.80, the lactate level (Lac) was 14.5 mmol/L, HGB and HCT could not be detected, and coagulation function continued to deteriorate (activated partial thromboplastin time (APTT) >170 sec, prothrombin time (PT) >120 sec, thrombin time (TT) >120 sec). When respiratory circulation became relatively stable, vascular embolization was performed immediately. Digital subtraction angiography (DSA) showed that a gastroduodenal artery pseudoaneurysm had developed. The gastroduodenal artery underwent microcatheter superselection. After successful superselection was confirmed by smoke, an appropriate volume of PVA500 particles was used to embolize the pseudoaneurysm-bearing artery. No pseudoaneurysm was observed in the follow-up angiography, and haemostasis was successful (Fig. 2). After the operation, the patient's blood pressure gradually stabilized, no tarry stool was observed, and blood gasses, CBC, coagulation and other indicators gradually returned to normal.

The patient suffered from severe upper gastrointestinal bleeding resulting in severe postoperative ischaemia and hypoxia and poor survival of skin grafts. After his general

condition improved, on the 18th day after admission, wound debridement and skin grafting were performed again. After the surgery, the wound was completely healed, and scar management and rehabilitation were performed (Fig. 3). After discharge, the patient was followed for 6 months. His growth and development were the same as his peers, and no further gastrointestinal bleeding occurred.

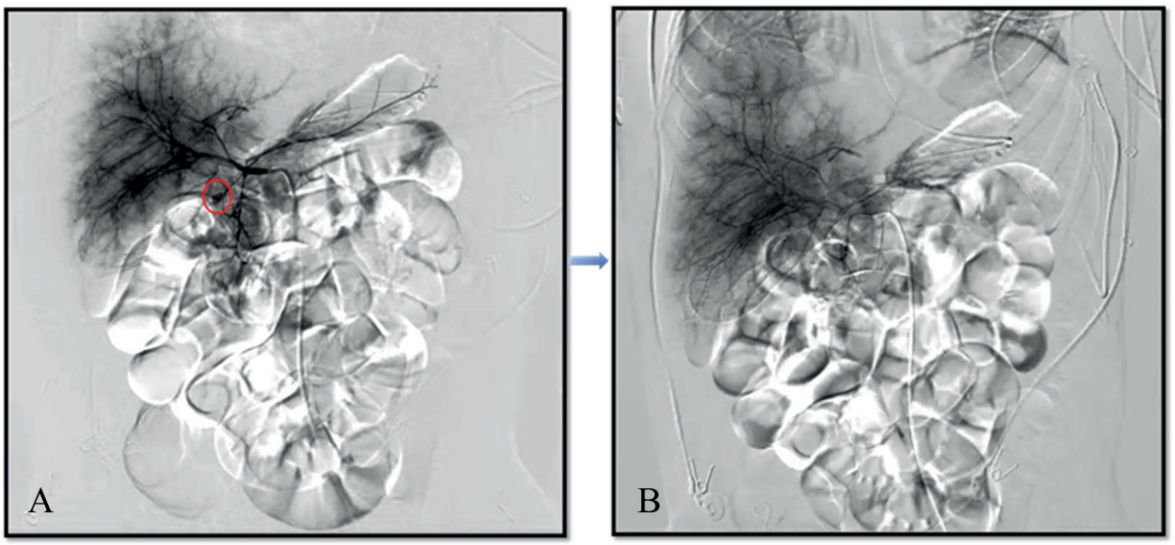


**Fig. 2.** DSA vascular embolization. A. Before embolization treatment: the red circle shows the bleeding site; B. After embolization treatment, there was no bleeding point. DSA: digital subtraction angiography.

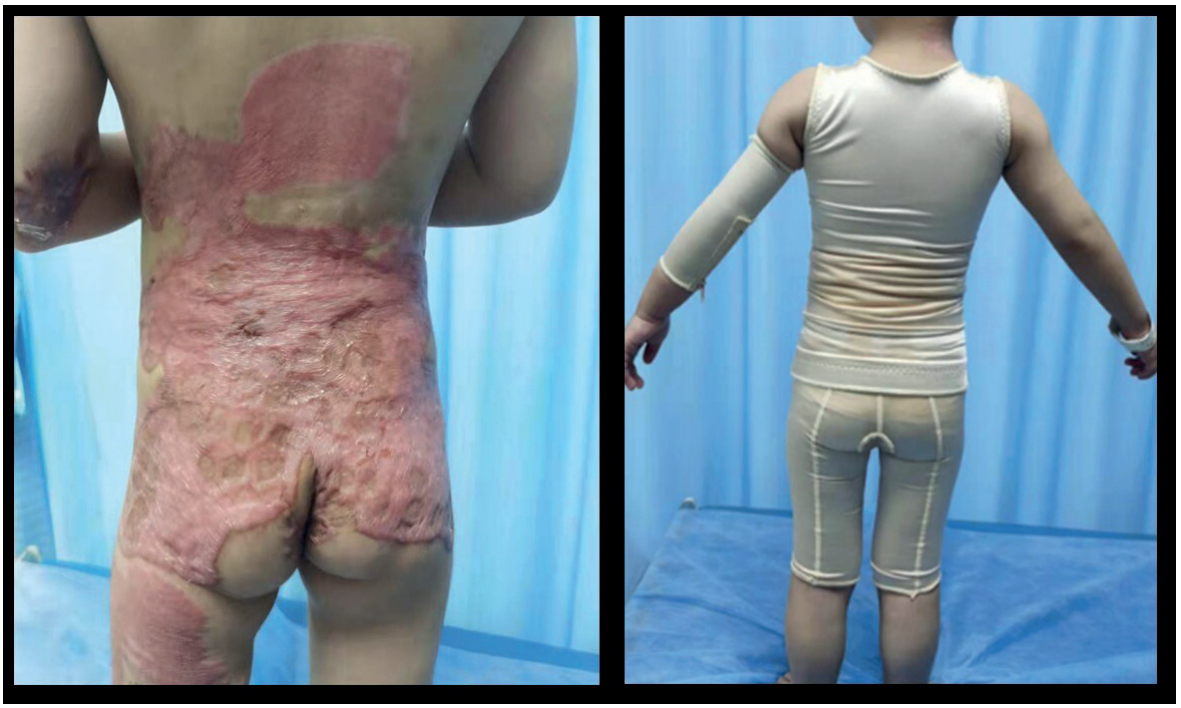

**Fig. 3.** Scar management and rehabilitation after wound healing.

## Discussion

Postburn stress ulcers are a common complication of burns and are prone to ulcerative bleeding, which increases treatment difficulty and prolongs the hospitalization of paediatric patients. The gastrointestinal tract of severe burn victims is not routinely examined by gastroscopy, so mucosal damage is often not detected before bleeding or perforation occurs.[5] The specific pathogenesis of postburn acute gastrointestinal ulcers may be due to gastric mucosal ischaemia–reperfusion injury caused by postburn body stress response. This leads to a decrease in the levels of prostaglandin E2 and bicarbonate, resulting in a decrease in the defence level of the gastric mucosa.[6] Additionally, a strong and excessive stress response will cause a series of damages to the body, including tissue decomposition and energy consumption, ischaemia and hypoxia, immune suppression and endocrine disorders.[7] After severe burns, a series of neuroendocrine reactions occur in the hypothalamic–pituitary–adrenal axis and the sympathetic nervous system, resulting in increased excitability of the vagus nerve.[8] A dysregulation of blood flow in the gastric mucosa under stress may also result from sympathetic nervous system activation.[9,10] In addition, after burns, the body is in a high metabolic state, with accelerated protein decomposition and insufficient protein synthesis[11], and the repair ability of the gastric mucosa is reduced, which further increases the risk of gastrointestinal bleeding. This patient was admitted to hospital for severe burns in multiple locations on the body, and his PCT level, which is considered to be related to body stress and the absorption of toxins from the wound, was significantly increased. Due to the child's poor self-regulation ability, the systemic stress response was severe, the infection progressed deeper into the wound due to improper postinjury wound treatment, and the child became increasingly prone to complicated gastrointestinal ulcers. In addition, debridement and skin grafting may have aggravated the body's stress response, leading to the occurrence of Curling's ulcer combined with massive haemorrhage.[12]

In addition, surgical stress may also be an aggravating factor for gastrointestinal bleeding in this child, but it is not the main factor. Firstly, the intraoperative and postoperative hemodynamics of the patient were in good condition. Secondly, laboratory testing carried out the night after surgery showed that coagulation function was normal. Furthermore, post-operative stress ulcers occurred late, between the eighth and the thirty-seventh day after the operation. Most likely due to the superposition of septicaemia on the systemic reaction following the operation.[13]

Gastrointestinal bleeding in children can easily result in coagulation dysfunction and organ dysfunction. Therefore, children with burns complicated with gastrointestinal bleeding should receive emergency treatment to avoid delays in recovery.[14] Bleeding in Curling's ulcer is mostly diffuse blood oozing from the canal wall, and active arterial haemorrhage is rare. However, in the child described in the present study, gastroscopy showed that the bleeding was not diffuse oozing from the intestinal wall but focal active bleeding in the duodenal bulb. This may have been related to the relative weakness of the child's duodenal bulb structure.[15,16] At present, treatment is still based primarily on pharmacological haemostasis or further haemostasis under gastroscopy. Interventional haemostasis is rare. Vascular DSA does not only locate the bleeding site but can also be used to perform embolization or drug perfusion treatment of the bleeding artery. The continuous development of DSA technology and coaxial microcatheters now enables microcatheters to be rapidly inserted into the proximal end of the bleeding artery for embolization or the perfusion of vasoconstrictor drugs to achieve effective haemostasis. Studies suggest that for patients with haemodynamic instability, those with failure or non-use of

endoscopic treatment and those in whom computed tomography angiography (CTA) cannot detect bleeding vessels, transcatheter embolization may be safer, showing a lower 30-day mortality.[17] When the patient in the present study presented gastrointestinal bleeding symptoms (continuous production of tarry black stool), haemostatic drugs, fluids and blood transfusions were administered through intravenous and gastric tube injections. At the same time, an MDT of representatives from the departments of gastroenterology, vascular surgery, paediatrics, intensive care medicine, general surgery and blood transfusion developed a stepwise treatment plan. The patient's condition deteriorated due to the ineffectiveness of the previous pharmacological haemostasis treatment. Emergency gastroscopy was performed to clarify the bleeding site. Due to the patient's small intestinal lumen, the intestinal wall was thin and could not be used for haemostasis under conventional gastroscopy. Subsequently, given the location of the bleeding, rapid and precise gastroduodenal arterial embolization was performed. Therefore, for paediatric burn patients with upper gastrointestinal haemorrhage, gastroscopy should be performed to confirm the bleeding site when pharmacological haemostatic treatment is ineffective, and if necessary, combining it with vascular embolization can improve the success rate of treatment.

Curling's ulcers combined with massive haemorrhage after burns in children are rare. They are characterized by a rapid onset, fast changes in the disease and extremely difficult treatment. An MDT that includes representatives from gastroenterology, interventional radiology, paediatrics, intensive care, general surgery and blood transfusion should be convened to discuss and develop a treatment plan. Full use of gastrointestinal endoscopy and interventional vascular treatment should be made to obtain the maximum benefit/risk ratio for patients and improve the success rate of treatment.

## Ethical approval

Written informed consent was obtained from the parent of the patient for publication of the patient's clinical details and clinical images.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: YW, HS; data collection: YW, YY; analysis and interpretation of results: YW, YY, ZS; draft manuscript preparation: YW, HS. All authors reviewed the results and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

# Clinical application of metagenomic next-generation sequencing in purulent meningitis: a case series

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## ABSTRACT

**Background.** Purulent meningitis remains an important cause of mortality and morbidity among children worldwide. An immediate diagnosis of the causative microorganism is critical to significantly improving the outcome of this condition.

**Case.** In this study, we collected cerebrospinal fluid (CSF) samples from four patients clinically diagnosed with purulent meningitis. Patients with purulent meningitis may present with a variety of clinical symptoms or laboratory results. Infectious microorganisms including *Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumonia,* and *Haemophilus influenzae* were identified in the CSF samples via metagenomic next-generation sequencing (mNGS).

**Conclusions.** mNGS is effective for the immediate detection of pathogens, which can in turn facilitate prompt diagnosis and treatment among individuals with purulent meningitis, especially if conventional CSF results (such as CSF culture and polymerase chain reaction) are negative.

**Key words:** purulent meningitis, metagenomic next-generation sequencing, pediatrics, diagnosis, case series.

Purulent meningitis is an inflammation of the meninges affecting the pia, arachnoid, and subarachnoid space caused by a purulent bacterial infection. It commonly has an acute onset and is characterized by high fever, headache, meningeal irritation, and other symptoms. The etiology and clinical characteristics of purulent meningitis with onset at different ages in children significantly differ.[1] Moreover, it is an extremely serious intracranial infectious disease. If timely treatment is not provided, it can be life-threatening or cause severe neurological sequelae. The morbidity of purulent meningitis in developed countries is 1.4–6.0 per 100,000 individuals, and the mortality rate is approximately 5.2% in newborns. The morbidity and mortality rates in developing countries are higher than those in developed countries, thereby showing multiple growth.[2-4]

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Due to a lack of knowledge about its causative pathogen, strain variation, and unreasonable application of antibiotics, purulent meningitis has a high mortality rate, and survivors present with severe neurological sequelae. Therefore, early diagnosis and timely administration of optimal antimicrobial therapy are important.

Metagenomic next-generation sequencing (mNGS) is a newly developed technology for the immediate, efficient, and unbiased collection of nucleic acid sequence information for all microorganisms. In 2014, a case of *Leptospira* infection was diagnosed via mNGS. Hence, its diagnostic value has been rapidly recognized.[5] A multicenter prospective study was conducted to investigate the efficacy of mNGS in the cerebrospinal fluid (CSF). In total, 204 patients with primary encephalitis, meningitis, or myelitis from eight hospitals in the United States were included in this research. Compared with conventional methods (such as CSF culture and polymerase chain reaction [PCR]), the positive and negative coincidence rates of mNGS were

80% and 98.2%, respectively. Further, 13 (22%) cases of infections were detected via mNGS alone.[6] Although mNGS is more expensive and takes longer to detect, it can simultaneously sequence billions of nucleic acid fragments using high-throughput technology, unlike traditional PCR tests, which require specific primers.[7,8] At present, mNGS has been increasingly used for the diagnosis of infectious diseases, particularly in cases with limitations in conventional diagnostic methods. Herein, we report four patients with complicated purulent meningitis in which the pathogens were identified via mNGS.

## Case

### *Case 1*

A 3-year-old boy was admitted to our hospital due to fever with an unknown cause and was diagnosed with acute lymphoblastic leukemia. After receiving chemotherapy and blood transfusion, his body temperature gradually normalized. One month after admission, the patient again presented with fever (temperature: 38.4°C), and he developed chills, headache, stiff-neck, and left upper gingival pain and redness with localized ulceration. *Pseudomonas aeruginosa* was isolated from blood and catheter cultures. He was further clinically diagnosed with gingivitis and sepsis. Treatment was switched to meropenem and micafungin, and chemotherapy was discontinued. Severe sepsis and septic shock subsequently developed, and the patient received fluid resuscitation, continuous positive airway pressure-assisted ventilation, and antimicrobial (meropenem, amikacin, and micafungin) therapy. However, the child was always drowsy, and he presented with a stiff-neck, grade 3 muscle strength of both upper limbs, and grade 2 muscle strength of both lower limbs. The routine blood tests revealed that the patient's C-reactive protein (CRP) and procalcitonin (PCT) levels were significantly elevated (CRP: 241 mg/L; PCT: 1.56 ng/ml). Brain magnetic resonance imaging (MRI) showed subdural effusion on the right frontotemporal top and brain tissue compression (Fig. 1). Hence, we needed to pay high attention to an intracranial infection. However, the routine CSF biochemical examination result was normal. The routine CSF bacterial culture (culture medium: 5% sheep blood agar and enriched chocolate agar, culture time: 72 h) and PCR had negative results. Furthermore, *P. aeruginosa* was detected in the CSF sample via mNGS (BGI Group [Beijing, China]). The patient was further diagnosed with purulent meningitis and subdural effusion. Antimicrobial therapy was continued. After treatment, the patient's vital signs stabilized, and his general condition improved. The patient was transferred to the department of pediatric hematology.

### *Case 2*

A 9-year-old boy underwent a cerebellar hemisphere lesion resection smoothly. The patient had a fever on day 2 of hospitalization (postoperative period), with the highest body temperature reaching 40°C. While on tracheal intubation, the boy was unconscious, and

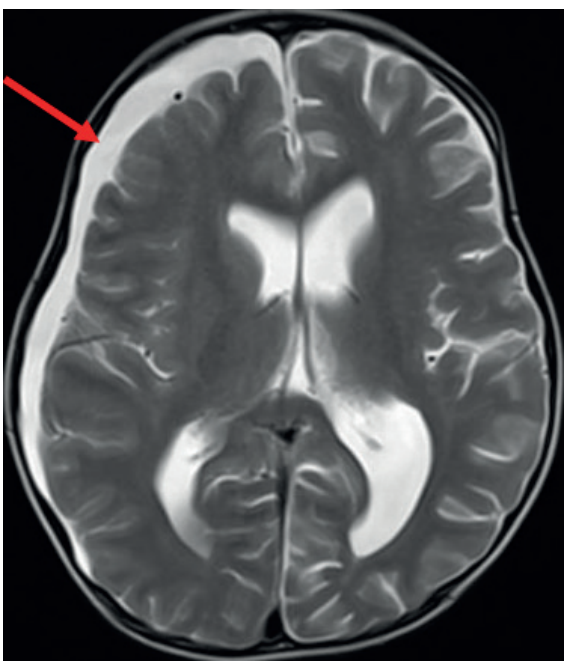

**Fig. 1.** Imaging changes of the brain in case 1.

he presented with occasional spontaneous breathing and anisocoria. Moreover, his pupils did not react to light. The boy had a persistent high fever, and he underwent multiple routine blood tests, which revealed a white blood cell (WBC) count of 24.78 × $10^9$/L with 91% neutrophils. The CRP and PCT levels were 214.53 mg/L and 7.68 ng/mL, respectively, and both values were significantly higher than normal. CSF analysis revealed that the total WBC count was 403.00 × $10^6$/L (with 74% multiple nuclear cells) and the red blood cell (RBC) count was 60.00 × $10^6$/L. The protein level was elevated at 1.66 g/L. Meanwhile, the CSF chloride level decreased to 117.7 mmol/L, and the CSF glucose level decreased to 4.14 mmol/L. The level of blood glucose was 6.0 mmol/L. Based on the test results, we considered the presence of an intracranial infection, which was treated with vancomycin and meropenem. However, the microbial diagnostic examinations, including repetitive routine CSF culture, blood culture and PCR, had negative results. Furthermore, the CSF sample was collected and sent for pathogen detection via mNGS, and the examination detected *Staphylococcus aureus*. Moreover, surgical pathological results showed pilocytic astrocytoma. During hospitalization, the boy's general condition was poor, with repeated and persistent high fever. Craniocerebral computed tomography (CT) scan revealed changes in the right cerebellar hemisphere, intracranial pneumatocele, left frontal epidural hematoma, hydrocephalus, right frontal subdural hematoma, and epidural hematoma after cranial surgery. The boy had severe brain damage. On the 30th day of admission, the boy's condition did not improve, and routine CSF examination was re-performed. CSF analysis revealed that the WBC count was 704.00 × $10^6$/L (with 56% multiple nuclear cells). The protein level was significantly elevated. Meanwhile, CSF glucose and chloride levels decreased. The CSF culture tested positive for *S. aureus*, and this was consistent with the result of mNGS upon admission. On the 33rd day of admission, the boy's condition still did not improve, and his family refused treatment.

***Case 3***

A 4-month-old-boy presented to the hospital due to complaints of fever for 4 days and intermittent convulsions with unconsciousness for 6 hours. The child was born at 35 + 5/7 weeks of gestation. His family history included seizures. A physical examination revealed poor response and drowsiness. Laboratory tests showed the following: WBC count, 15.69 × $10^9$/L; 79% neutrophils; CRP level, 132.49 mg/L; and PCT level, 0.554 ng/mL. The CSF pressure was 160 mm$H_2O$, and the CSF looked like rice soup. The CSF test revealed that the WBC count was 19617.00 × $10^6$/L and the RBC count was 100.00 × $10^6$/L. CSF analysis revealed that the glucose, chloride, and protein levels were 0.13 mmol/L, 114.5 mmol/L, and 1.59 g/L, respectively. The level of blood glucose was 5.5 mmol/L. Brain MRI revealed bilateral frontotemporal subarachnoid space widening, which can indicate subdural effusion or extracerebral hydrocephalus. With consideration of the available information, the patient was diagnosed with purulent meningitis and subdural fluid. Then, the patient was treated with vancomycin combined with meropenem. The patient's CSF sample was immediately taken to BGI Group (Beijing, China) for mNGS. The result showed that *Streptococcus pneumonia* was detected in the CSF sample. Then, the CSF and blood culture results indicated the presence of *S. pneumoniae*, which was consistent with the results of mNGS. The patient continually received vancomycin treatment, and meropenem was discontinued. On the 8th day of admission, the state of the child gradually improved with intermittent fever and occasional convulsions. Electroencephalogram (EEG) results were abnormal with slow background activity and predominantly (multiple) sharp slow waves in the bilateral posterior temporal regions. Treatment with oral topiramate for epilepsy was added to the regimen. On the 36th day of admission, brain CT scan showed bilateral frontotemporal subdural effusion, right frontotemporal and subdural hematoma, hydrocephalus, and periventricular edema. Hence, the supplementary clinical

diagnosis was hydrocephalus. Compared with previous findings, the recent CT scan result showed aggravation. Considering that the child had a serious intracranial infection complicated with hydrocephalus, subdural effusion, and hematocele, there might be neuro-related sequelae. The family chose to transfer the patient to another hospital for treatment, and we then lost contact with the patient's family.

### *Case 4*

A 9-year-old girl was admitted to the hospital due to a 2-day history of headache, fever, and vomiting accompanied by seizures for 4 hours. She received ceftazidime for 2 days prior to admission. Physical examination showed that her vital signs were stable while she was comatose. Her Glasgow Coma Scale score was 9. Both eyelids, more prominently on the left side, were drooping. She had double vision and was not able to move her eyeballs. The muscle strength of both upper limbs was grade 3, and the tendon reflexes were weak. The muscle strength of both lower limbs was grade 0, and the tendon reflexes were absent. The sensory plane was located at the level of the bilateral groin. Laboratory blood tests showed that the WBC count was $40.05 \times 10^9$/L with 92% neutrophils. The CRP level was 220.58 mg/L, and the PCT level was 30.69 ng/mL. Both values were higher than normal. CSF analysis revealed that the total WBC count was $29374.00 \times 10^6$/L and the RBC count was $300.00 \times 10^6$/L. The protein level was elevated at 1.70 g/L. Meanwhile, the CSF glucose level (1.26 mmol/L) and chloride level (116.7 mmol/L) decreased. The level of blood glucose is 4.5 mmol/L. A brain CT scan revealed that the cerebral falx and cerebellar tentorium had a higher density, with consideration of adenoid hypertrophy and sinusitis. Therefore, the patient was diagnosed with purulent meningitis and was immediately treated with meropenem, intrathecal dexamethasone, and vancomycin. The CSF and blood cultures did not detect any microorganisms and PCR examinations were also negative. The CSF and blood samples were collected and sent for pathogen detection via mNGS. Results showed the presence of *Haemophilus influenzae* in the CSF and blood. Therefore, vancomycin was discontinued, and amikacin was added as an antimicrobial treatment. MRI of the thoracic spine revealed a suspected abnormal signal shadow in the spinal cord at the 7th thoracic vertebrae to the 1st lumbar vertebrae. An additional clinical diagnosis of myelitis was made, and antimicrobial treatment, immune modulation, and other therapies were continued. The patient's clinical symptoms improved gradually. She had clear consciousness and no fever. However, the muscle strength was still abnormal. On the 74th day of admission, the patient had a significant improvement in her clinical condition and laboratory test results. Further, there were no abnormalities on thoracic and lumbar spine MRI. Her condition improved, and she was then discharged from the hospital.

## Discussion

Purulent meningitis is a common disease with sudden onset and has a high mortality rate in the pediatric population. Timely diagnosis and appropriate antibiotic therapy are effective in achieving complete recovery. This report showed the identification of pathogens via mNGS in four patients with purulent meningitis. Notably, mNGS was found to have an important role in the early identification of pathogens particularly among patients with critical illnessess.

Purulent meningitis is one of the most serious infections in childhood, and is associated with serious complications. In this report, there were multiple types of complications including subdural effusion, hydrocephalus, brain abscess, and myelitis. These could lead to severe permanent sequelae if not treated promptly. The main clinical signs of purulent meningitis are fever, change in consciousness, vomiting, convulsion, and headache.[9] Epileptic seizures are the most common clinical symptom of acute-stage purulent meningitis. Purulent meningitis accompanied by acute epileptic

seizures may be a risk factor for epilepsy and nervous system sequela or mortality.[10,11] Yang et al.[12] showed that the rate of abnormal EEG patterns in patients with purulent meningitis was relatively low. Pomeroy et al.[13] monitored the EEG of 58 children with purulent meningitis accompanied by an acute epileptic attack. In case 3 of the current study, the patient presented with convulsions and abnormal brain waves, and antiepileptic drugs were provided. However, due to late complications including hydrocephalus, subdural effusion, and hematocele, nervous system sequelae may develop. Therefore, children with purulent meningitis should be diagnosed promptly, and appropriate and full-course antibiotic treatment should be provided immediately to reduce the development of complications. Meanwhile, in recent years, the proportion of typical cases has been gradually decreasing, and treatment based on experience can lead to misdiagnosis and delayed management. Therefore, in the diagnosis and treatment of purulent meningitis, accurate treatment must be provided based on the pathogen.

Conventional testing methods used in clinical microbiology laboratories include PCR, culture, and CSF antigen and antibody detection. Although these methods are currently applied, they have limitations in detecting the range of pathogens, particularly those that are uncommon. PCR has been a great advancement in numerous individual techniques that specifically target organisms. However, a rare organism can still be missed, or limited primers containing mismatches to the microbial strain involved will be used, which decreases the sensitivity of detection.[14] CSF culture is the gold standard for diagnosing purulent meningitis. The Infectious Diseases Society of America guidelines state that if acute bacterial meningitis is suspected, a sample must be obtained immediately for blood culture, and lumbar puncture for CSF biochemical examination, routine examination, and culture should be performed to confirm the diagnosis.[15] Nonetheless, a positive culture result is challenging to obtain due to the administration of broad-spectrum or prophylactic antimicrobial drugs prior to lumbar puncture, as well as the presence of organisms that are fastidious or slow growing.[16] The specificity of CSF bacterial culture is up to 97%. However, the sensitivity is only 25%–90%.[17] Due to the early application of antibiotics, the lowest positive rate of CSF culture was only 5.3%.[18] Meanwhile the yield of CSF culture in suspected cases is also low.[19] In the current study, patients 1 and 4 received antimicrobial drugs prior to lumbar puncture, and they had negative CSF culture results. The pathogenic factor of purulent meningitis is pathogen infection, and its nucleic acid is often detected in the CSF or brain tissue. Hence, it is theoretically feasible to sequence the metagenome of the CSF.

In recent years, mNGS has gradually been applied in the diagnosis of clinical infectious diseases. In our setting, we sent samples of blood and CSF to BGI Group (Beijing, China) for mNGS. BGI Group conducted mNGS as described previously[20], collecting 2-3 mL sample. They then extracted DNA following standard procedures. DNA libraries were constructed through DNA fragmentation, end repair, adapter ligation, and PCR amplification. They sequenced the qualified libraries by using the BGISEQ-100 platform.[21] They screen high-quality sequencing data and exclude low-quality reads, then performed computational subtraction of human host sequences mapped to the human reference genome (hg19) using Burrows-Wheeler alignment.[22] They classified the remaining data and simultaneously aligning the sequences to microbial genome databases for bacteria, viruses, fungi, and parasites downloaded from the US National Center for Biotechnology Information (ftp://ftp.ncbi.nlm.nih.gov/genomes). Experts work together to assess the patient's condition and interpret the mNGS results to identify possible etiologic agents. Finally, we identified the causative agent through mNGS.

Compared with conventional detection methods, mNGS has several advantages. For

example, it is not based on traditional culture methods and can detect pathogenic bacteria in samples after the application of antibiotics. In a multicenter study conducted by Wilson et al.[6], 32 (55.17%) of 58 patients were diagnosed with intracranial infection via mNGS. Thus, the positive rate of mNGS in purulent meningitis was significantly higher than that of traditional etiological detection. Of the four patients in this study, only one had a positive CSF culture result. Meanwhile, all patients underwent mNGS to confirm pathogenic infection. Table I shows the causative microorganism in four cases. This is primarily attributed to the fact that mNGS detection is an unbiased calculation of information about microbial DNA fragments, which is not associated with bacterial survival. Unlike traditional bacterial culture, a certain number of living bacteria is required. Therefore, whether antibiotics are used before sampling has little influence. All patients presented with complex purulent meningitis with different characteristics. That is, in case 1, the patient was immune deficient, and he developed purulent meningitis caused by sepsis. mNGS has evident advantages in the detection of different complex purulent meningitis. Unlike bacterial culture, which requires up to 72 h of final identification, the detection time of mNGS is short.[23] Clinicians can get timely pathogenic results via mNGS, which can improve clinical treatment level. The mNGS results of our patients were obtained after 1 day. In cases 4, antibiotic therapy was modified. Nevertheless, culture is still the gold standard and should not be neglected, because it alone allows for an antibiogram.

Several types of bacteria cause purulent meningitis. *S. pneumoniae*, *N. meningitidis*, *H. influenzae*, and *Listeria* are the most common pathogens of community acquired purulent meningitis.[17,24-26] In addition to the common pathogenic bacteria, *Coliform bacillus*, *Deform bacillus*, *Enterococcus*, and *P. aeruginosa* can cause purulent meningitis. mNGS can find both common pathogenic bacteria and other uncommon pathogenic bacteria. In case 1, *P. aeruginosa* infection was identified. *P. aeruginosa* is an opportunistic pathogen and the main pathogen of nosocomial infection. The patient presented with acute lymphoblastic leukemia, and the routine CSF biochemistry had normal results. However, the CRP level was high, and subdural effusion was observed. The CSF culture result was negative, and *P. aeruginosa* infection was confirmed via mNGS. Therefore, mNGS has an advantage in detecting pathogenic bacteria in purulent meningitis. However, it also has disadvantages. That is, mNGS is associated with a risk of contamination by environmental species during the routine collection of clinical samples. This can then result in misdiagnoses. In clinical diagnosis and treatment, mNGS detection results must be used in combination with clinical data, imaging characteristics, and routine laboratory test findings to confirm the presence of pathogenic, colonization, and contaminated bacteria.

In conclusion, the current series showed that mNGS could be used to diagnose purulent meningitis. Nevertheless, this preliminary finding should be examined in diagnostic trials in the future.

**Table I.** Culture, PCR, and mNGS result of of the four patients.

| Case | Culture | | | PCR | | mNGS | | |
|---|---|---|---|---|---|---|---|---|
| | Blood | CSF | Time | CSF | Time | Blood | CSF | Time |
| 1 | *P. aeruginosa* | negative | 3d | negative | 1d | Not tested | *P. aeruginosa* | 1d |
| 2 | *S. Aureus* | negative | | negative | | Not tested | S. Aureus | |
| 3 | *S. pneumoniae* | *S. pneumoniae* | | negative | | Not tested | *S. pneumoniae* | |
| 4 | negative | negative | | negative | | *H.influenzae* | *H.influenzae* | |

CSF: cerebrospinal fluid, mNGS: metagenomic next-generation sequencing, PCR: polymerase chain reaction,
CSF: cerebrospinal fluid, *P. aeruginosa: Pseudomonas aeruginosa, S. Aureus: Staphylococcus aureus;*
*S. pneumoniae: Streptococcus pneumonia, H.influenzae: Haemophilus influenzae.*

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**Ethical approval**

The First Hospital of Jilin University Institutional Review Board approved this study, with the need for informed consent waived.

**Author contribution**

The authors confirm contribution to the paper as follows: study conception and design: MD, CY; data collection: MD; analysis and interpretation of results: MD, CY, YL; draft manuscript preparation: MD, CY. All authors reviewed the results and approved the final version of the manuscript.

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The authors declare the study received no funding.

**Conflict of interest**

The authors declare that there is no conflict of interest.

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Case Report

# Pediatric appendicular actinomycosis: a case report and literature review

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## ABSTRACT

**Background.** Actinomycosis (ACM) is a rare infectious granulomatous disease caused by Actinomyces, a Gram-positive, filamentous, saprophytic bacteria. There are several types of pediatric ACM, such as orocervicofacial (55%) and other less common forms: abdominopelvic and thoracic. We report a case of a 16-year-old who presented with abdominal ACM in the setting of acute appendicitis. After the case report, we provide a short literature review of pediatric appendicular ACM cases published.

**Case.** A 16-year-old boy presented with nausea, vomiting, pain in the upper part of the abdomen and fever (37.5°C) lasting for 24 hours. On physical examination, the patient's epigastrium and lower right abdominal quadrant were tender. White cell count and C-reactive protein (CRP) were elevated at 16,300/μL and 48.6mg/L respectively. Ultrasonography (US) showed appendicolith and edema of the appendiceal wall, focally with stratification as well as periappendiceal inflammation. The patient underwent a classic appendectomy, and the postoperative course was without complications. Histopathological analysis showed diffuse transmural neutrophilic infiltration of the appendix, focally with areas of necrosis and abscesses. There were numerous brightly eosinophilic colonies made of filamentous bacteria, located predominantly in submucosa. Special stains Grocott-Gomori's Methenamine Silver and Gram were positive and a diagnosis of ACM was made.

**Conclusions.** Although appendicitis is very common in the general population, appendicitis associated with ACM is very rare, accounting for 0.02% - 0.06%, especially in the pediatric population. Diagnosis can be very challenging because they usually present with non-specific symptoms, and can form masses that mimic malignancies. Although rare, clinicians and pathologists should be aware of this entity. Satisfactory results and complete cure are achieved with adequate antibiotic therapy and surgery. In most cases, if there are no associated diseases, early and accurate diagnosis ensure an excellent prognosis.

**Key words:** appendix, actinomycosis, children.

Actinomycosis (ACM) is a rare infectious granulomatous disease, with an incidence between 1/300,000 and 1/1,000,000. ACM in the pediatric population is very rare, accounting for 3% of all ACM cases. It is caused by Actinomyces, a Gram-positive, filamentous, saprophytic bacteria. The most common cause of ACM in the human population is Actinomyces israelii.[1-3] There are several types of pediatric ACM such as orocervicofacial (55%) and other less common forms: abdominopelvic (20%) and thoracic (15%).[1]

In this paper, we report the case of a 16-year-old who presented with abdominal ACM in the setting of acute appendicitis. We also provide a short literature review of pediatric appendicular ACM cases published. We used MEDLINE database literature, and the search was performed using the PubMed service.

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Search terms: actinomycosis, appendix, children and pediatric were used in various combinations. Pediatric (0-18 years) cases with confirmed appendicular ACM were included.

## Case Report

A 16-year-old boy presented with nausea, vomiting, pain in the upper part of the abdomen and fever (37.5°C) lasting for 24 hours. On physical examination, the patient's epigastrium and lower right abdominal quadrant were tender. There were no predisposing factors or associated illnesses that could be linked to the current condition. White cell count and C-reactive protein (CRP) were elevated at 16,300/μL (12,300/μL granulocytes, 2,900/μL lymphocytes) and 48.6 mg/L respectively. A blood sample was obtained for blood culture, but it yielded negative results. Ultrasonography (US) showed appendicolith and edema of the appendiceal wall, focally with stratification as well as periappendiceal inflammation. Mesenteric lymph nodes of ileocecal region were enlarged, up to 16 mm. After preoperative preparations, according to the protocol, the patient underwent a classic appendectomy. Initial drug treatment consisted of the intravenous administration of antibiotics (amikacin and ceftriaxone) and analgesics. The postoperative course was without complications and the wound healed well. Control laboratory analyzes were within the reference range. Histopathological analysis of the appendix showed diffuse transmural neutrophilic infiltration, focally with areas of necrosis and abscesses (Fig. 1, Fig. 2). There were numerous brightly eosinophilic colonies made of filamentous bacteria, located predominantly in the submucosa (Fig. 3). Special stains Grocott-Gomori's methenamine silver and Gram were positive and a diagnosis of ACM was made (Fig. 4, Fig. 5). Amoxicillin was continued to be administered to the patient for the following six months after the diagnosis of ACM. The follow-up period lasted for one year, and no

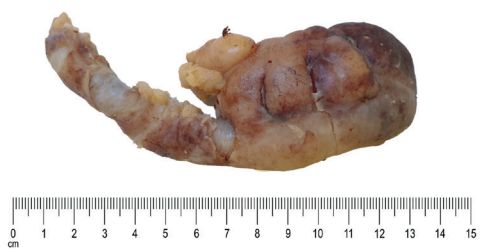


**Fig. 1.** Enlarged, inflamed appendix with fibrinous depositions on the serosal surface.

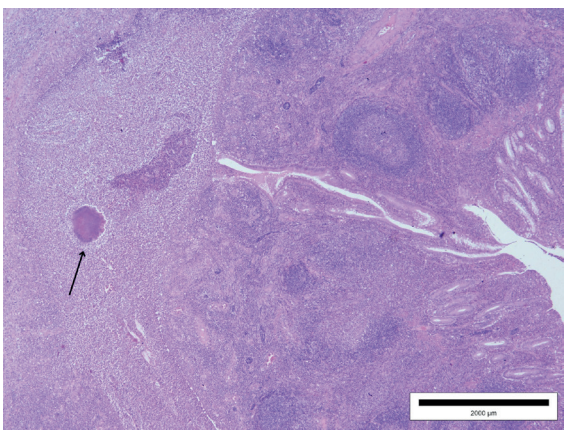


**Fig. 2.** Appendiceal wall with diffuse polymorphonuclear inflammatory infiltrate. Arrow pointing to oval eosinophilic mass in the submucosa (Actinomyces colony). (H&E, x40)

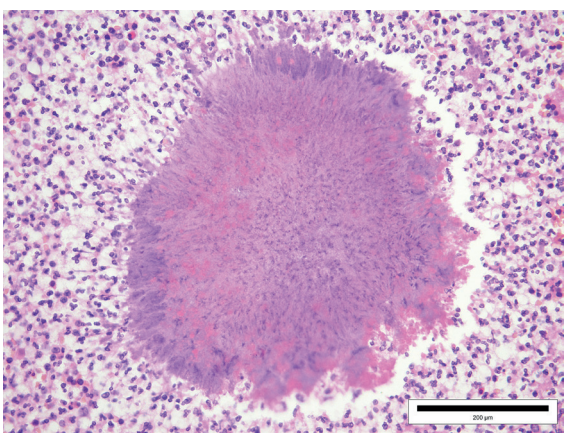


**Fig. 3.** Colony of Actinomyces rimmed by eosinophilic proteinaceous material (Splendore-Hoeppli). (H&E, x400)

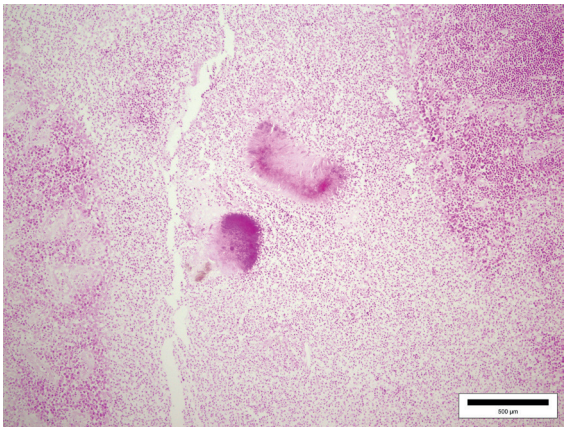

**Fig. 4.** Two colonies of actinomyces admixed with polymorphonuclear inflammatory infiltrate (Gramm stain, x100).

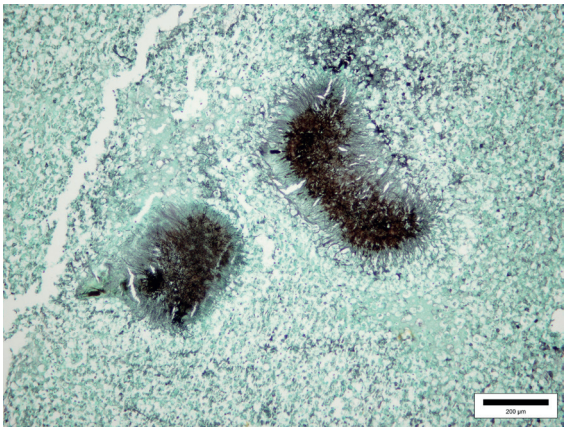

**Fig. 5.** Grocott-Gomori's Methenamine Silver stain depicts filamentous bacteria (Actinomyces). (x200)

complications were recorded. Informed consent for publication of this case report was obtained from the patient's family.

## Discussion

Actinomyces are obligatory anaerobic, commensal bacteria of the oral, gastrointestinal and urogenital mucosa.[4] In most cases, actinomyces enter the tissues through small mucosal defects, usually during pathologic processes that disrupt the integrity of the mucosa such as appendicitis in our case.[4] Abdominopelvic ACM can be associated with diverticulitis, other intestinal diseases such as Crohn's disease or ulcerative colitis, trauma, recent surgery and intrauterine contraceptive devices. Factors predisposing the development of infection are alcoholism, diabetes, immunosuppression etc.[1,5] Despite all the aforementioned, according to a systematic review conducted by Manterola et al.[4], no contributing factor was identified in 57.9% of articles associated with ACM, which is consistent with our findings.

Of patients with abdominopelvic ACM, 66% have ileocecal region involvement.[6] Although very common in the general population, appendicitis associated with ACM is very rare accounting for 0.02% - 0.06% of the cases, especially in the pediatric population.[7] Diagnosis of these infections can be very challenging. They usually present with non-specific symptoms lasting for several months such as abdominal pain, fever, weight loss etc. The most common symptoms of abdominal ACM in the pediatric population are pain and a palpable lump.[1] Also, ACM can form masses mimicking malignancies, often with suppurative granulomatous inflammation which results in fistula formation.[5] There are plenty of complications related to ACM such as abscess formation with draining fistulas, osteomyelitis, meningitis, brain abscess, endocarditis and disseminated ACM.[4]

ACM is usually underdiagnosed due to the empirical application of antibiotics and specific anaerobic conditions needed for cultivation (only 17.9% of ACM cultures are positive).[4] Preoperative diagnosis of ACM is only 10%.[6] Computerized tomography (CT) and magnetic resonance imaging (MRI) are useful methods for determining the exact location of the inflammation. Those methods can also depict sinus and fistula tracts often associated with ACM, but they are not specific enough. Fine needle aspiration (FNA) or core biopsy are still useful diagnostic procedures for the confirmation of CT and MRI suspected lesions.[8] Laboratory findings are non-specific, they may indicate anemia, leukocytosis with elevated neutrophils and elevated values of

inflammatory parameters such as CRP, as in our case.[5] Colonoscopy findings are also non-specific, showing normal, thickened or ulcerated mucosa, appendicular inversion or nodular button like lesion.[9]

There are a wide variety of differential diagnoses for ACM. Due to its infiltrative pattern, ACM usually mimics neoplasms and Crohn's disease of the ileocecal region.[9-12] In the majority of cases, the correct diagnosis was determined postoperatively, following histopathological analysis. On standard hematoxylin and eosin staining ACM is characterized by sulfur granules made of densely packed filamentous bacteria outlined by eosinophilic material made of immunoglobulins called the Splendore-Hoeppli phenomenon.[13]

ACM is usually treated with high doses of antibiotics such as beta lactams (penicillin) often for a long period, between 6 and 12 months. Also, ACM is sensitive to erythromycin, minocycline, doxycycline and clindamycin, which is very important in cases of allergic reactions to beta lactams.[5,14]

Table I summarizes the published pediatric cases with confirmed appendicular ACM.[13-19] One of the most prominent differences between our case and other cases listed in Table I were the duration of the symptoms and the formation of a pseudotumorous mass. Unlike the other cases listed in the table, where a macroscopically visible pseudotumorous mass or phlegmonous inflammation of the appendix involving surrounding organs was observed in six out of seven cases, such findings were not present in our case. We presume that such findings are due to the short duration of the illness in our case, only 24 hours, and the pseudotumorous mass did not have time to develop in contrast to most cases listed in Table I where symptoms of the disease appeared several weeks or even months prior to surgery.

Appendicular ACM often presents a diagnostic challenge as it can mimic the appearance of a tumor or other noneoplastic conditions in the ileo-cecal region across different age groups. It is crucial to differentiate between conventional appendicitis and appendicular ACM, as the treatment approach and duration differ significantly, with ACM requiring a much longer course of therapy.[20,21] Furthermore, it should be noted that not every case of ACM presents with the formation of a mass or follows a long course of the disease, as demonstrated in our case. Although rare, it is important for clinicians and pathologists to be aware of this condition. In a majority of cases, when there are no associated diseases, an early and accurate diagnosis, followed by appropriate treatment, ensures an excellent prognosis.

## Ethical approval

Informed consent was obtained from the patient's family for publication of this case report and accompanying images.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: MM, RJ, MĐ, LjS, NP, JJ; data collection: MM, RJ, MĐ, LjS, NP, JJ; analysis of results: MM, RJ, MĐ, LjS, NP, JJ; draft manuscript preparation: MM, RJ, MĐ, LjS, NP, JJ. All authors reviewed the results and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that there is no conflict of interest.

**Table I.** Appendicular actinomycosis in the pediatric population.

| Case author, year, reference no. | Sex, age (years) | Signs and symptoms | Symptom duration | WBC (/μL); CRP (mg/L) | Imaging methods and findings | Diagnosis HP; culture | Surgery | Medication |
|---|---|---|---|---|---|---|---|---|
| Schmidt P, et al. 1999. (15) | F, 15 | pain nausea mass | 2 days | 11,200; 98 | US: traget sign, RLQ | + ; - | appendix and cecum resection | tetracyclines (penicillin alergy) |
| Campo JM, et al. 2001. (16) | M, 11 | fever pain mass | 3 months | NA; 108 | CT: RLQ mass, hydronephrosis | + ; - | appendix and mass resection | IV PG, OP (12M in total) |
| Sumer Y, et al. 2004. (17) | F, 17 | pain fever mass | 2 months | 12,700; NA | US, CT: RLQ mass, hydronephrosis | +; NA | *en bloc* excision of the mass | IV PG 4W, amoxicillin 6M |
| Yiğiter M, et al. 2007. (14) | M, 13 | pain nausea mass | 4 weeks | RR | US, CT: RLQ mass | +; NA | appendectomy | IV PG 2W, OP 6M |
| Liu V, et al. 2010. (1) | F, 13 | pain nausea fever constipation mass | 3 weeks | 14,700; NA | CT: RLQ mass | +; NA | appendectomy | IV PG 3W, OP 10W |
| Karakuş E, et al. 2014. (1) | M, 14 | pain vomiting | NA | 10,400; 35,6 | US: appendicitis | +; NA | appendectomy | NA |
| Completo S, et al. 2022. (13) | M, 9 | pain mass | 5 months | 17,800; 65 | US: appendicitis | +; NA | appendectomy | IV PG 1M, amoxicillin 12M |

CRP: C-reactive protein, CT: computerized tomography, F: female, HP: histopathology, IV PG: intravenous penicillin G, M: male, M: months, NA: not available, OP: oral penicillins, RLQ: right lower abdominal quadrant, RR: within the reference range, US: ultrasound, W: weeks, WBC: white blood cells.

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Case Report

# Pneumatosis cystoides intestinalis mimicking free intraabdominal air following chemotherapy for relapsed acute myeloblastic leukemia in a transplanted neutropenic child: a case report

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## ABSTRACT

**Background.** Pneumatosis cystoides intestinalis (PI) is a rare but important condition in which widespread air sacs are found in the submucosa, and subserosa of the bowel wall. Although it has several etiologies, children receiving chemotherapy are at risk for PI. Preferred imaging tools for the diagnosis are abdominal direct radiography and computed tomography. In patients with PI, rupture of intramural air sacs is the source of benign pneumoperitoneum, causing free air without true intestinal perforation. Intestinal perforation or obstruction are indications for surgical intervention.

**Case.** Here, we present a 4-year-old patient diagnosed with acute myeloblastic leukemia (AML), who underwent allogeneic hematopoietic stem cell transplantation (HSCT) from a matched sibling donor (MSD) and developed PI after HSCT. The patient was consulted to the pediatric surgery department, and her oral feeding was stopped. Broad spectrum antibiotics (teicoplanin, metronidazol and vancomycin) were initiated. Her fever increased during the 24-hour monitoring, there was no stool passage, CRP (>25 mg/dL, normal value <1 mg/dL) and abdominal distension increased and there was prolonged neutropenia and radiologic investigations could not rule out intestinal perforation, so the patient underwent exploratory laparotomy. No intestinal perforation was found. There was no sign in the intestinal wall and numerous gas-filled cysts of various sizes.

**Conclusions.** PI is an uncommon complication, and direct radiography/computed tomography scans are very helpful in making the diagnosis in suspicious cases. PI, should be kept in mind, especially in transplanted or relapsed leukemia patients receiving intensive chemotherapy.

**Key words:** leukemia, pneumatosis intestinalis, surgery, transplant.

Pneumatosis intestinalis (PI) is a rare condition characterized by the presence of gas within the mucosal and submucosal layers of the intestinal wall.[1] Although its etiology is still unknown, many factors including the production of intraluminal bacterial gas are considered to be responsible for the pathogenesis.[2] It is considered that gastrointestinal tract permeability increases with the mucosal damage caused by chemotherapy, prolonged neutropenia and interfering infections which leads to the diffusion of gas into the gut wall.[3] Several chemotherapeutic agents including cytarabine have been shown to trigger the mechanism.[4] PI can be asymptomatic, but it may also manifest itself with abdominal pain and distension.[5] Radiologic diagnosis can be made with direct radiography in two thirds of the cases. However, computed tomography (CT) is necessary to make a diagnosis in one third of the cases.[6] While a conservative approach

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is possible, emergency surgery may also be necessary. Here, we report a case with acute myeloblastic leukemia (AML) who developed PI following treatment with Flag-Ida because of a relapse following allogeneic hematopoetic stem cell transplantation (allo-HSCT).

## Case Report

A four-year-old girl diagnosed with AML underwent matched sibling donor allo-HSCT from her sister due to an early relapse of AML. The patient was routinely monitored from the outpatient clinic under immunosuppressive treatment for graft-versus-host disease (GVHD) prophylaxis without any problems. On the 104th day following the transplant, the patient experienced a second relapse and received a 5-day Flag-Ida chemotherapy regimen consisting of fludarabine (30 mg/m$^2$, days 1-5), cytarabine (2 g/m$^2$, days 1-5), idarubicin (10 mg/m2, days 3-5) and intrathecal therapy (methotrexate 12 mg, dexamethasone 4 mg, cytarabine 30 mg) for the induction of remission. In order to avoid neutropenia, granulocyte colony-stimulating factor was given daily at 5 mcg/kg. The patient had fever and abdominal pain 13 days after the chemotherapy was stopped and routine laboratory tests, abdominal ultrasonography (USG), chest and upright direct abdominal X-rays were performed to investigate the etiology of neutropenic fever. USG was normal except for minimal splenomegaly. Free intraperitoneal air was detected following an upright abdominal X-ray (Fig. 1). Her vital signs were stable except for a fever of 38ºC. Physical examination revealed only mild abdominal swelling. There was no abdominal guarding or rebound, and the bowel sounds were unremarkable. There was no sign of peritonitis. Stool passage was normal and there was no history of nausea and vomiting. Laboratory work-up revealed a leucocyte count of 1×10$^9$/L, neutrophil rate of 87%, a platelet count of 52×10$^9$/L, and a hemoglobin level of 10.2 g/dL. Electrolytes, liver and renal function tests, lipase, amylase, and arterial blood gases were normal. CRP was 23 mg/dL (N <0.1) and other laboratory results were likewise within normal limits.

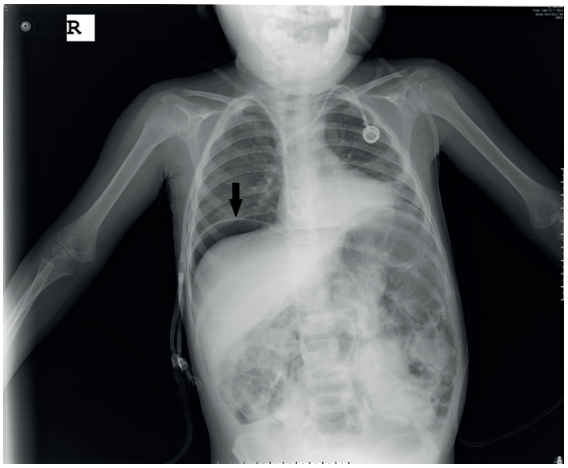


**Fig. 1.** Image of free air below the diaphragm on the right in the direct X-ray (black arrow).

Written informed consent was obtained from the patient's parents for the publication of this case report.

## Management & Outcome

Abdominal CT was performed, and in addition to intramural gas along the colon, diffuse free intraperitoneal air was detected (Fig. 2). After the patient was consulted to the pediatric surgery department, her oral feeding was stopped. The stomach was decompressed with a nasogastric tube. Intravenous fluid therapy was initiated in accordance with a conservative approach. Broad-spectrum antibiotics (imipenem, amikasin, linezolid) were used in the first week as neutropenic fever treatment in immunosuppressive patients, and then shifted to teicoplanin, metronidazole, levofloxacin, cefepime and finally meropenem and vancomycin. However, her fever increased during the 24-hour monitoring period, there was no stool passage, CRP (>25 mg/dL, normal value <1 mg/dL) and abdominal distension were increased, and there was prolonged neutropenia and radiological investigations could not rule out intestinal perforation, so the patient underwent exploratory laparotomy.

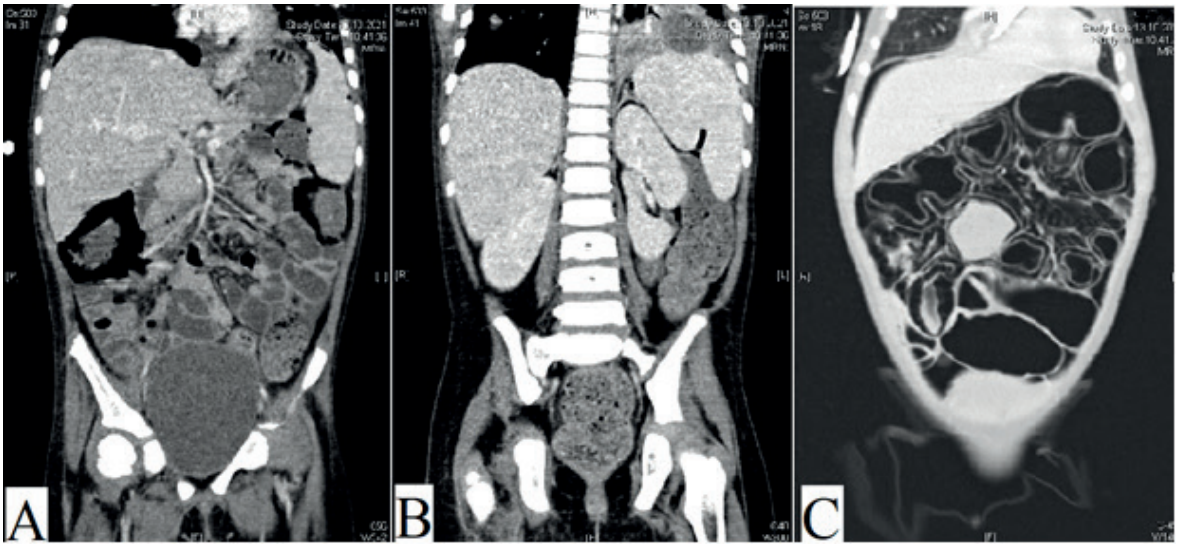


**Fig. 2.** Pneumoperitoneum can be seen on the abdominal computed tomography (CT) images (A, B). Abdominal CT image shows intramural and extramural gas of the ascending, transverse and descending colon in lung window (C).

No intestinal perforation was found. There was no sign in the intestinal wall, and numerous gas-filled cysts with sizes varying between 0.3 mm and 2 cm were observed on the serosa of ileal loops, leading to the diagnosis of PI (Fig. 3). No positive results were obtained from microbiological analyses (Salmonella, Shigella, Yersinia, or Campylobacter in blood culture and stool). A clostridium difficile toxin test resulted as negative.

Following a 21-day course of treatment, the abdominal distension gradually resolved. Abdominal CT and direct radiography revealed that PI had completely disappeared. In the meantime, neutropenia also resolved. CRP value regressed to 2 mg/dL. However, increases were seen in white blood cell (WBC), lymphocyte and monocyte count in the fourth week of follow-up. Peripheral smears revealed a myeloblast percentage of 25%. These findings led to a bone marrow aspiration, which revealed that the disease was not in remission. Currently, the patient is hospitalized and monitored at the hematologic service of our clinic. Following bone marrow remission, we plan to perform a haploidentical stem cell transplantation.

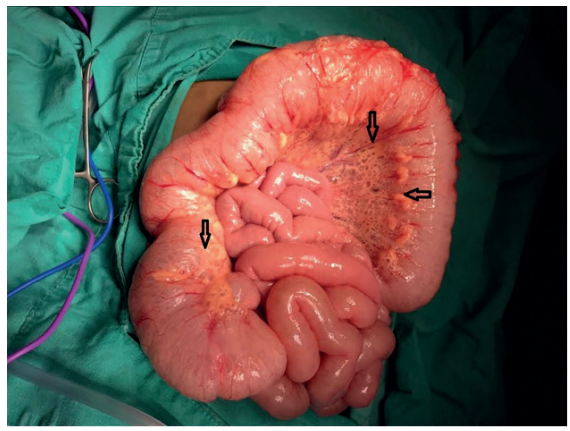

**Fig. 3.** Intraoperative image showing gas-filled cysts on the colon serosa of varying sizes (black arrows).

## Discussion

PI is a rare clinical condition characterized by multiple gas-filled cysts in the subserosal and submucosal layers of the bowel.[7] Although the underlying pathology is not clear, many explanations have been proposed. The most emphasized hypothesis is that pressure increases due to the gas released from intestinal bacterial proliferation in a neutropenic patient following cytotoxic chemotherapy, disruption of the mucosal barrier of the

bowel, and subsequent gas accumulation in the submucosal area.[4] In addition, the myeloablative chemotherapy regimen given for HSCT and immunosuppressive therapy for GVHD prophylaxis, which will contribute to the prolongation of neutropenia, can also be considered risk factors for PI.[8] A conditioning regimen, which consists of busulfan, cyclophosphamide, and melphalan, was performed on our patient. The disease relapse of our patient in the first 3 months of the post-transplant period while she was taking immunosuppressive agents, including tacrolimus, mycophenolate mofetil, and methylprednisolone, for chronic cutaneous severe GVHD; with all these findings and just 13 days after the administration of chemotherapy were considered important factors for the occurrence of PI. The literature shows that there is an increased incidence of PI in children undergoing allo-HSCT or receiving GVHD prophylaxis.[9,10] In addition, a recent study conducted by Wallace et al.[11] concluded that systemic steroid use was associated with an increased incidence of PI in 990 consecutive pediatric transplant recipients.[11] Further, PI may develop as a result of several gastrointestinal system diseases, such as appendicitis, inflammatory bowel diseases, pyloric stenosis, necrotizing enterocolitis, ulcers and following endoscopic procedures.[7] Usually diseases that involve the colon can affect all regions of the small intestine.[12] There was isolated colon involvement in our case. The cecum, colon ascendens, colon descendens, and, partially, the sigmoid colon were affected. Radiological investigations-abdominal CT (more sensitive) and direct radiography– are quite valuable for diagnosis. In addition to enabling diagnosis in most PI cases, the above-mentioned investigations also provide a differential diagnosis regarding acute abdomen cases requiring emergency surgery. Both investigations show a low-density linear or bubbly pattern of gas in the bowel wall. Although abdominal CT suggested PI in our case, the general condition of the patient and the severity of the clinical picture necessitated laparotomy.

Although there is no established treatment yet, surgery is not the first choice in asymptomatic cases because spontaneous resolution is observed in 50% of cases. Favorable outcomes have been achieved in these cases with close follow-up and supportive therapy including bowel rest, total parenteral nutrition, and antibiotics.[13] The clinical status and symptoms of the patient should also be taken into consideration. Deterioration of the general condition of the patient, destabilization of vital signs, suspicion of peritonitis and intestinal perforation probability seem to be the indications for the surgical intervention.[14] Initially, we approached our case conservatively since the course of her disease was stable, abdominal distension was absent and stool passage was normal, but surgical intervention was performed after her fever rose to 39ºC, abdominal distension occurred and stool passage stopped. In the case series of Li et al.[3] including 514 pediatric patients with AML and acute lymphoblastic leukemia who developed PI, it was concluded that most of the cases could be managed with a conservative approach. In the same study, they found that USG was less sensitive than direct X-ray in making the diagnosis, similar to our case. The presence of febrile neutropenia in the patient and the complex clinical picture caused by the sepsis that occurred following intensive chemotherapy can be considered to have also played a role in the decision to operate in our case.

In conclusion, PI is a rare condition that can be seen in patients who receive chemotherapy for hematological and oncological malignancies. Direct radiography and CT are very helpful in making the diagnosis in suspicious cases. Patients can be managed conservatively with bowel rest and intravenous broad-spectrum antibiotics, and surgical intervention can be needed during follow-up.

## Ethical approval

Written informed consent was obtained from the patient's parents for the publication of this case report.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: UA, BŞK, BA; data collection: UA, ÖÖ, KT, İŞ; analysis and interpretation of results: UA, İŞ, KT; draft manuscript preparation: BA, BŞK, ÖÖ. All authors reviewed the results and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

# ORAI1 defect in a patient with disseminated CMV infection and severe hypotonia

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## ABSTRACT

**Background.** A clinical presentation similar to severe combined immunodeficiency (SCID) with defective T cell activation but normal lymphocyte development occurs due to certain molecule defects including *ORAI1*- and *STIM1*.

**Case.** A four-month-old girl sufferd from fever, restlessness, diarrhea, and poor weight gain following the neonatal period. There was consanguinity and a positive family history. She had hypotonia and spontaneous opisthotonic posture. Refractory and extensive CMV infections were detected; immunological investigations revealed normal quantitative immunoglobulins and low numbers of CD3+, CD4+, and CD8+ cells. The next generation sequencing analysis revealed a mutation in the *ORAI1* gene.

**Conclusions.** The present patient's history of refractory and widespread CMV infections shows a clinically substantial reduction in resistance against opportunistic microorganisms. This case emphasizes the importance of considering STIM1 and ORAI1 defects in patients with SCID phenotype and neurologic involvement, such as hypotonia.

**Key words:** primary immunodeficiency diseases, CMV infection, ORAI1 deficiency.

Primary immunodeficiency diseases (PIDs) are inherited defects of the innate or adaptive arms of the immune system that lead to an increase in the incidence, frequency, or severity of infections, malignancies, and immune dysregulation.[1] The most severe form of primary immunodeficiency, needing immediate care, is severe combined immunodeficiency (SCID). T and B cell development/function are defective in SCID, and cellular and adaptive immune responses are impaired. In the early months of life, patients present with severe infections caused by viral, fungal, and bacterial agents.[2]

Despite normal lymphocyte development and number, a clinical presentation similar to SCID occurs due to certain molecule defects leading to compromised T cell activation. Calcium is a critical second messenger essential for lymphocyte and non-immune cell activation. Stromal interacting molecule (STIM) 1 and STIM2 found in the endoplasmic reticulum (ER) membrane and ORAI1 are the critical molecules in the development of Ca2+ channels (CRAC) which is a crucial step of signal transduction and lymphocyte activation. The amount of ER

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Ca2+ controls the CRAC channel. Its opening results in Ca2+ influx or store-operated Ca2+ entry (SOCE). SOCE is an essential Ca2+ signaling pathway in lymphocytes necessary for activating several Ca2+-dependent enzymes and transcription factors that regulate immune cell development, proliferation, and function.[3]

ORAI1- and STIM1-deficient patients have a clinical phenotype that is similar to SCID, with recurrent and chronic infections, autoimmunity, ectodermal dysplasia, aberrant enamel development, and muscular hypotonia.[4] In a review; congenital myopathy was observed in all patients with ORAI1 mutations. Generalized muscular hypotonia resulted in poor head control, delayed ambulation, and a positive Gower's sign.[5]

Here, we report an infant with a mutation in the *ORAI1* gene who presented with CMV infection and hypotonia.

## Case Report

A four-month-old female patient presented with complaints of fever, restlessness, diarrhea, and poor weight gain after the neonatal period. Diarrhea was watery, non-bloody, and 8-10 times a day. There was parental consanguinity and a sibling death during infancy due to diarrhea. Physical examination revealed fever (38.8°C), irritability and cachexia. Weight and height were 4250 g (<3p) and 60 cm (10p), respectively. She had pale skin, cutis marmoratus on the legs, retrognathia, and a high-arched palate. She had axial hypotonia, no head control, and spontaneous opisthotonic posture. Object tracking was defective. Deep tendon reflexes were normoactive without clonus. During the evaluation for chronic diarrhea, we detected a high serum cytomegalovirus (CMV) viral load (316.000 copies/ml). Echocardiography showed left ventricular hypertrophy and myopericarditis. Upper endoscopy and colonoscopy showed a normal mucosal appearance the hematoxylin-eosin staining of the colonic mucosa revealed viral inclusion bodies in the lamina propria; and immunohistochemical studies showed CMV antigen positivity. We could not test CMV PCR from the colon tissue biopsy. Bone marrow aspiration was performed for prolonged fever and showed a high CMV viral load (398,471 copies/ml). Brain magnetic resonance imaging (MRI) showed mild inferior vermian hypoplasia, elongated midbrain, thin corpus callosum, and symmetric T2 hyperintensity along with restricted diffusion in the central tegmental tracts of the pons (Fig. 1).

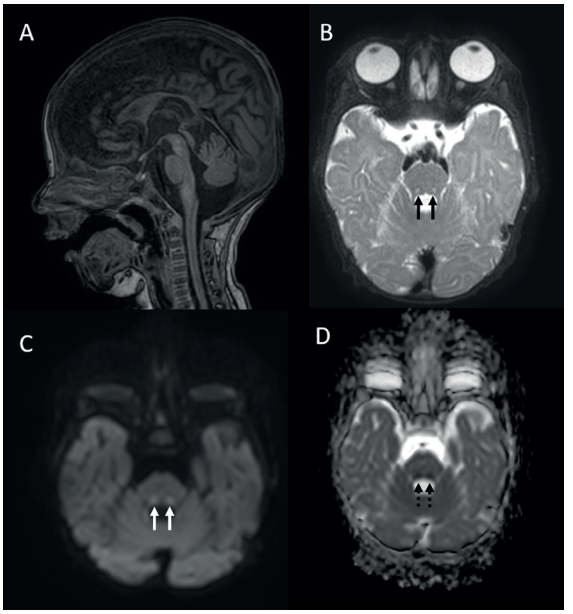


**Fig. 1.** Brain MRI of the patient at 4 months-old. Sagittal T1-weighted (W) image (A) shows mild inferior vermian hypoplasia, elongated midbrain and thin corpus callosum. Axial T2-W image (B) shows bilateral T2 hyperintensity of central tegmental tracts in the pons (arrows) and diffusion restriction as demonstrated by hyperintensity on trace diffusion (C) and low ADC values on ADC map (D).

Regarding PID, immunological investigations revealed normal quantitative immunoglobulins and low numbers of CD3+, CD4+, and CD8+ cells (Table I). Since she had low levels of serum uric acid levels and T cell counts, we analyzed the purine nucleoside phosphorylase (PNP) enzyme activity, and it was normal.

**Table I.** Immunological evaluation of the patient

| Parameters | On admission | Reference values |
|---|---|---|
| Complete blood count | | |
| Hemoglobin (g/dl) | 8.0 | 9.5-13.5 |
| WBC (/mm$^3$) | 6,600 | 6,700-14,000 |
| ANC (/mm$^3$) | 1,540 | 1,000-7,000 |
| ALC (/mm$^3$) | 4,000 | 3,900-9,000 |
| AEC (/mm$^3$) | 80 | 100-1,000 |
| Trombocytes (/mm$^3$) | 382,000 | 150,000-450,000 |
| Immunoglobulins (mg/dl) | | |
| IgA | 93.9 | 13.5-72 |
| IgG | 1,320 | 294-1,165 |
| IgM | 47.9 | 33-154 |
| Total IgE (IU/L) | 2.88 | 0-65 |
| Anti HBs (mIU/ml) | 173.64 | |
| Lymphocyte subpopulations (% and absolute counts /mm$^3$) | | |
| CD3+ | 38% | 51-77 |
| | 1,406 | 2,500-5,600 |
| CD3+CD4+ | 28% | 35-56 |
| | 1,036 | 1,800-4,000 |
| CD3+CD8+ | 11% | 12-23 |
| | 407 | 590-1,600 |
| CD16+56+ | 10% | 3-14 |
| | 370 | 170-830 |
| CD19+ | 50% | 11-41 |
| | 1,850 | 430-3,000 |
| CD45RA | 82% | |
| CD45RO | 17% | |
| TCR $\alpha\beta$ | 35% | |
| TCR $\gamma\delta$ | 2% | |
| Lymphocyte activation test | | |
| CD3 | 36% | 59.1-80.7 |
| CD4 | 25% | |
| CD25 | 30% | 86.9-99.8 |
| CD69 | 21% | 61.2-91.8 |
| CD3+CD25+ | 28% | 52.4-93.7 |
| CD4+CD25+ | 19% | |
| CD3+CD69+ | 17% | 47.9-84.8 |
| CD4+CD69+ | 12% | |

AEC: absolute eosinophil count, ALC: absolute lymphocyte count, ANC: absolute neutrophil count, Ig: immunoglobulin, WBC: white blood cell.

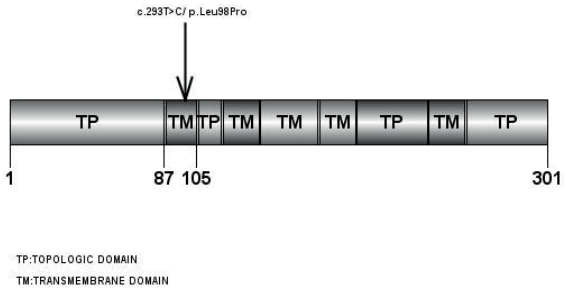


**Fig. 2.** Representation of the ORAI1 gene and the patient's mutation.

The patient had low lymphocyte activation and proliferation test results compared to the control, therefore she was diagnosed with combined immunodeficiency. Subsequently, we started her monthly immunoglobulin replacement therapy and gave gancyclovir and CMV hyper immunoglobulin therapies for CMV infection. Despite treatment, serum CMV copy number increased (1,373,511 copies/ml). The patient died at the age of five months owing to aspiration pneumonia.

After her death, we detected a homozygous mutation in the *ORAI1* gene (c.293T>C/p.Leu98Pro) by the next-generation sequencing PID panel analysis (Fig. 2). The Sanger sequencing and family segregation of the genetic defect confirmed the variant and we provided genetic counseling to the family.

An informed consent was received from the family for the manuscript.

## Discussion

We here describe an infant who presented with a combined immunodeficiency phenotype with hypotonia and disseminated CMV infection. This case highlights STIM1 and ORAI1 defects in patients with SCID phenotype and neurologic involvement, such as hypotonia.

With four transmembrane domains, ORAI1 functions as a plasma membrane protein. Various types of human tissues and cells express ORAI1[5] required for the growth and function of skeletal muscle, eccrine sweat gland function, and tooth enamel calcification.[6] ORAI1 gene mutations have been associated with combined immunodeficiency and myopathy, as well as recurring severe infections caused by viral, bacterial, mycobacterial, and fungal pathogens, resulting in pneumonia, meningitis, enteritis, and sepsis.[4]

Our patient presented with heart, bowel, and bone marrow involvement of CMV infection. As gancyclovir, we administered CMV hyper immunoglobulin therapy to the patient. Although CMV infection in healthy children and adults is usually mild or asymptomatic, immunocompromised individuals are at risk of more severe disease including pneumonia, hepatitis, neutropenia, thrombocytopenia and enterocolitis.[7] Hematochezia and diarrhea are the most common symptoms of gastrointestinal invasive CMV (GI-CMV) infection.[8] Wetwittayakhlang et al.[9] reported that the presenting symptoms of GI-CMV may differ in immunocompromised and immunocompetent patients. Besides the typical finding of GI-CMV, gastrointestinal bleeding is less frequent in immunocompromised patients, although the disease was more extensive in that study. GI-CMV can be diagnosed with cytopathologic changes (owl-eye appearance) demonstrated by hematoxylin-eosin staining or CMV antigen identified by immunohistochemistry. Mucosal punched-out ulcers are the most prominent finding in endoscopic evaluation. However, relatively milder mucosal manifestations such as diffuse or focal erythema and edema are more common in immunocompromised patients.[9] Our patient with non-bloody diarrhea showed no endoscopic mucosal abnormality but positive histopathological findings of CMV colitis. Hence, tissue biopsy is required to exclude GI-CMV in immunocompromised patients.

Central nervous system (CNS) manifestations of cytomegalovirus (CMV) infection include meningitis, retinitis, encephalitis, and myeloradiculitis. These unusual manifestations occur almost exclusively among severely immunocompromised patients.[10] There was

no evidence of CMV infection on brain MRI in the present patient. However, central nervous system involvement is common in patients with PIDs and may be due to several factors, including infections and autoimmunity, and a direct result of defective genes. Neurologic involvement may occur as an initial manifestation of some of these conditions and may account for morbidity and mortality of affected patients.[11] To our knowledge, brain MRI findings of patients with ORAI1 deficiency are not present in the literature. Central hypotonia detected in our patient may be related to the structural abnormalities in brain MRI.

Myopathy in ORAI1- and STIM1-deficient patients becomes apparent soon after birth as global, nonprogressive muscular hypotonia with reduced muscle strength and endurance like in the presented patient. Peripheral hypotonia is the expected clinical finding in patients with ORAI1 deficiency due to myopathy, initial manifestations are insufficient head control and a general reduction in muscle tone.[12] We do not have a muscle biopsy to comment on the presence of such muscle involvement in our patient, clinical findings suggested central hypotonia. Myasthenia-induced recurrent respiratory tract infection is a poor prognostic factor in severe patients.[13] Our patient had a swallowing disorder related to hypotonia resulting in aspiration pneumonia and death.

Hemophagocytic lymphohistiocytosis (HLH) may be seen in ORAI1 deficiency due to a failure in lymphocyte cytotoxicity.[14] Our patient had anemia and recurrent fever but did not fulfill the criteria for HLH. Four ORAI1 deficiency patients, all having enamel hypoplasia, hypocalcified amelogenesis imperfecta, anhidrosis, and ectodermal dysplasia, were reported, and three novel biallelic mutations were detected in these four patients.[15] Recurring fever in our patient might be attributed to anhydrosis or recurrent infections.

In patients with *ORAI1* defect, as in our patient, lymphocyte count may be appropriate according to age-matched levels, as in the present patient. Immunoglobulin levels are generally variable. However, T cell proliferation is insufficient in response to mitogen and antigens. Although we start anti-microbial and immunoglobulin replacement therapy for patients with ORAI1 deficiency to prevent infections, hematopoietic stem cell transplantation (HSCT) is the only curative therapy.[16] Unfortunately, compatible family donor identified for HSCT was lacking, and the patient died while unrelated donor screening was ongoing.

In conclusion, refractory and disseminated CMV infections imply a substantial decline in defense against opportunistic microorganisms. Even though supportive treatments temporarily improve clinical symptoms, they are insufficient to prevent disease progression. The current case demonstrates the critical significance of evaluating ORAI1 deficiency in individuals with combined immunodeficiency and severe infantile hypotonia. In PIDs, the clinical history and physical examination results are the essential signals that guide patients to the diagnosis. Neonatal screening for PIDs may help the early diagnosis of these patients.

## Ethical approval

Informed consent was obtained from the family for the publication of the case report.

## Author contribution

The authors confirm contribution to the paper as follows: study conception and design: KD, SE, HNB; data collection: KD, SE, HNB, MO; analysis and interpretation of results: SE, DY, KKO, HHG, IE, DC, IT; draft manuscript preparation: KD, SE, DY, HHG, KKO. All authors reviewed the results and approved the final version of the manuscript.

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## Conflict of interest

The authors declare that there is no conflict of interest.

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Case Report

# Dyskinesia due to mexiletine overdose: a rare presentation

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## ABSTRACT

**Background.** Mexiletine, a class IB antiarrhythmic, is a structural analog of lidocaine. Our knowledge of mexiletine overdose is based on lidocaine overdose reports. Only a few cases of mexiletine overdose have been reported, including fatal overdoses. Mexiletine toxicity primarily affects the central nervous, cardiovascular, and gastrointestinal systems.

**Case.** A 16-year-old female was brought to our hospital by ambulance after taking an unknown dose of mexiletine in a suicide attempt. Ventricular fibrillation developed while in the ambulance; cardiopulmonary resuscitation was started and spontaneous circulation returned within 1 min. The patient had been taking oral mexiletine for 1 month to treat primary erythromelalgia. Her vital signs were normal, but she was unconscious. Following gastric lavage she was transferred to the pediatric intensive care unit. Midazolam and levetiracetam were required due to uncontrolled seizures. During the first hour of hospitalization, severe dyskinesia characterized by abnormal involuntary large hyperkinetic movements in all 4 extremities was observed and successfully treated with 2 doses of intravenous biperiden. The patient was discharged on day 6 of hospitalization.

**Conclusions.** Mexiletine overdose can be life-threatening. In addition to rapid and effective resuscitation, rapid identification and management of cardiovascular and central nervous system manifestations are key to preventing morbidity and mortality. The presented case had severe dyskinesia that was successfully treated with repeated doses of biperiden. Biperiden did not cause arrhythmia. Based on the presented case, we think biperiden should be considered for the treatment of movement disorders in cases of mexiletine overdose.

**Key words:** biperiden, dyskinesia, intoxication, mexiletine, poisoning.

Mexiletine, a class IB antiarrhythmic, is a structural analog of lidocaine, but with higher oral bioavailability, and is, therefore, administered orally.[1] Mexiletine can be used to treat neuropathic pain and is also reported to be beneficial for erythromelalgia.[2,3] Our knowledge of mexiletine overdose is based on reported cases of lidocaine overdose.[1,4-7] Both drugs have similar effects, but the literature includes only a few cases of mexiletine overdose.[1,4-7] Mexiletine toxicity primarily affects the central nervous system, cardiovascular system, and gastrointestinal system.[1] The possible clinical findings of mexiletine overdose include dizziness, visual impairment, paresthesia, dysarthria, ataxia, memory loss, euphoria, agitation, confusion, lethargy, coma, disorientation, psychosis, tremors, muscle twitches, seizures, respiratory arrest, arrhythmia and hypotension, and nausea and vomiting.[1] Fatal cases of mexiletine overdose have also been reported.[5,7] Herein we present a case of dyskinesia due to mexiletine overdose that was successfully treated with biperiden.

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## Case Report

A 16-year-old female was brought to our hospital by ambulance after taking an unknown dose of mexiletine in a suicide attempt. Ventricular fibrillation developed while in the ambulance; cardiopulmonary resuscitation was started while preparing for defibrillation and spontaneous circulation returned within 1 min, without the need for defibrillation. The patient had been taking oral mexiletine for 1 month to treat primary erythromelalgia. Her parents reported that the patient took 16 mexiletine pills; however, 50 pills were missing from the pill packets. The parents reported that they did not think the patient to took any other drugs.

Upon hospital admission the patient's vital signs were normal, but she was unconscious with a Glasgow coma score of 9. Blood cell count, and liver and kidney function tests were normal. Blood gas analysis showed mixed acidosis with pH 7.16 and partial carbon dioxide pressure of 56 mmHg, lactate level of 73 mg $dL^{-1}$, and bicarbonate level of 16 mmol $L^{-1}$. Gastric lavage was performed, but no drugs were noted. A bolus of sodium bicarbonate (1 mEq $kg^{-1}$) was administered. In addition, 2 boluses of midazolam were required due to generalized tonic-clonic seizures that occurred 10 min after admission. The patient was transferred to the pediatric intensive care unit, and levetiracetam was loaded. As the patient's seizures recurred frequently, midazolam infusion was started. Severe dyskinesia characterized by abnormal involuntary large hyperkinetic movements in all 4 extremities was observed during the first hour of hospitalization; therefore, 2 mg of intravenous biperiden was administered and the dyskinesia improved rapidly. A second dose of biperiden was required, as dyskinesia recurred 2 hours later; after the second dose dyskinesia did not recur. On day 2 of hospitalization the patient was fully conscious and midazolam infusion was withdrawn following 24 hours of no seizures. Electrocardiography, electroencephalography, and brain magnetic resonance imaging were normal. Following a psychiatric evaluation the patient was discharged on day 6 of hospitalization. The patient's parents provided written informed consent to have their daughter's case published.

## Discussion

Patients with mexiletine overdose, and severe central nervous system or cardiovascular manifestations should be followed-up in the intensive care unit, as in the presented case. Moreover, removal of the drug via gastric lavage and activated charcoal should be considered.[1] Mexiletine is rapidly metabolized, highly protein-bound, and extensively distributed to tissues; therefore, it is not expected to be removed from the body via hemodialysis or other extracorporeal methods.[1] Although it was reported that dialysis might be beneficial, it was not considered in the presented case. [8] Gastric lavage was performed in the presented case, but we were unsure whether the drug could be removed.

Arrhythmias, including sinus bradycardia, sinus arrest, atrioventricular nodal or ventricular rhythms, heart blocks and asystole can occur in cases of mexiletine overdose.[1] Bradyarrhythmia that causes hypotension can be managed with isoproterenol infusion; however, heart blocks are common, and pacemaker implantation may be required.[4,5] In such cases extracorporeal life support should be a consideration.[4,5] Sodium bicarbonate is beneficial in cases of massive overdose with prolongation of the QRS interval based on the electrocardiography.[1] As the presented case had ventricular fibrillation, sodium bicarbonate treatment was administered.

Prolonged seizures do not usually occur in cases of lidocaine overdose; however, seizures after mexiletine overdose have been frequently reported.[1,4,9] Because mexiletine has a long redistribution time and prolonged presence in the gastrointestinal tract, its toxicity is expected to persist longer than that of lidocaine.[4] In the presented case benzodiazepine infusion and levetiracetam were required for seizures.

Dyskinesia is a rare side effect of mexiletine.[10] A study on the side-effects of mexiletine in 40 patients reported that only 1 had dyskinesia and the drug was discontinued.[10] To the best of our knowledge the present case report is the first to describe dyskinesia following mexiletine overdose.

Biperiden is an anticholinergic drug that can successfully treat dyskinesia.[11] It did not cause arrhythmia or other side-effects while providing very rapid improvement in the clinical condition of the presented case.

Mexiletine overdose can be life-threatening. Rapid identification and management of cardiovascular and central nervous system manifestations are key to preventing morbidity and mortality. In the presented case severe dyskinesia was successfully treated with biperiden; therefore, we think biperiden should be considered for treating movement disorders in cases of mexiletine overdose.

### Ethical approval

Written informed consent for patient information to be published was provided by the patient's parents.

### Author contribution

The authors confirm contribution to the paper as follows: study conception and design: ZO, NT; data collection: ZO, OA; draft manuscript preparation: ZO, İB, RMY, HAÇ. All authors reviewed the results and approved the final version of the manuscript.

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### Conflict of interest

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**Short Communication**

# Gender affirming care is an evidence-based approach and misinformation harms patients and clinicians

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On August 12th, 2023, two Turkish newspapers, namely 'Yeni Şafak' and 'Aydınlık', published articles about a scientific paper published in an international peer-reviewed journal. In the newspaper article, they misrepresented the research findings of this scientific paper involving results of a study undertaken by a team of Turkish academics on the provision of gender affirming care for young people. The newspaper article promoted a false narrative about academicians providing gender affirming care, accusing them with misconduct, deviation from scientific principles, and engaging in unethical practices.[1]

The treatment and guidance provided by the authors was evidence based practice.[2] Over 25 of the most prestigious and eminent medical organizations have acknowledged the need of medical treatment for gender dysphoria, and recognize that access to gender-affirming care for gender diverse youth is best practice, most importantly life-saving. These include the World Health Organization, the American Academy of Pediatrics, the American Academy of Child and Adolescent Psychiatry, the Endocrine Society and the Society for Adolescent Health and Medicine.[3]

There is a growing body of evidence indicating that the provision of gender-affirming treatment to young individuals experiencing gender incongruence is associated with enhanced physical and mental health outcomes.[4] Puberty suppression, a therapy approach that is both safe and fully reversible, is one of the most important advances in the history of transition treatment.[2] Numerous studies have demonstrated the efficacy of this intervention in mitigating the progression of permanent and psychologically stressful alterations linked to biological puberty. Moreover, it has been found to yield quantifiably improved outcomes in terms of psychosocial functioning and overall quality of life.[5]

Gender-diverse youth are more susceptible to experience depression, anxiety, familial rejection and victimization, social isolation, and are more likely to engage in nonsuicidal and suicidal self-harm and substance use, potentially as a result of external hostile influences.[6] Compassionate care provided by clinicians who are knowledgeable about gender diversity has been shown to reduce these risks,[3] but gender-affirming care providers, who are already limited in number in our country,[7] have expressed concern regarding how the aforementioned article portrays gender-affirming care and its providers. Articles of this nature have the potential to limit the ability and number of clinicians willing to practice gender affirming care in accordance with evidence-based standards as they promote verbal, physical, and emotional abuse against physicians.

This type of targeted harassment is not unique to our country. A very recent study by Hughes et al.[8] examined the experiences of gender-

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affirming care providers in the United States and discovered that 70% of participants had received threats related to providing gender-affirming care and described how this impacted their psychological well-being. This type of provocation only serves to further stigmatize such youth and their families, which eventually restricts their access to health care, and worsens their health outcomes. We call on policy makers, institutional leaders and health professionals to do all within their power to support gender diverse youth, their families, and the clinicians who serve them.

### Author contribution

The authors confirm contribution to the paper as follows: Manuscript draft SA and ZAÖ. Both author approved the final version of the manuscript.

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### Conflict of interest

The authors declare that there is no conflict of interest.

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## CASE REPORTS



## SHORT COMMUNICATION



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